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PATHOLOGY

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Phase Microscopy in the Practice of Pathology
H. Russell Fisher

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J. J. Bocian and A. E. Geschke

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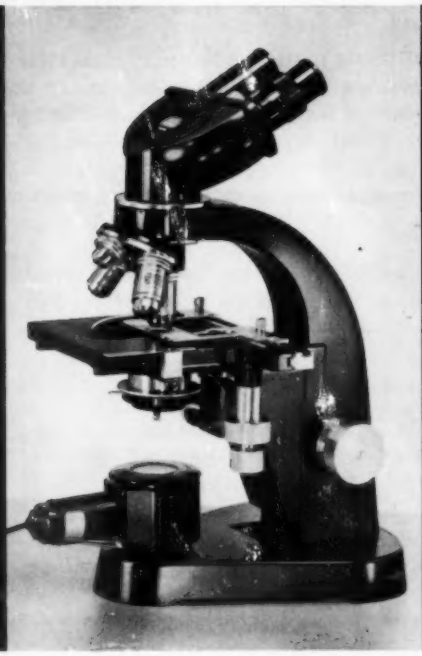

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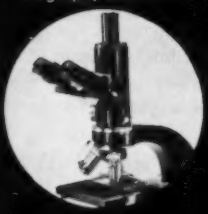

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Phase Microscopy in the Practice of Pathology

H. RUSSELL FISHER, M.D., Los Angeles

The sphere of the practice of pathology is usually delineated by the methodology of the laboratory, in which instrumentation is of prime importance. Even in this day of electronic quantitation, most of us, having spent half of our professional life peering through its optical system, would choose the microscope as the most basic and essential instrument available to the pathologist. Bright-field microscopy has been supplemented during the last two decades by significant new techniques, including polarization, fluorescent, phase, and electron microscopy. Each is of proven importance as a research tool. Among them, phase microscopy holds the greatest promise of having protean application in the routine procedures of the everyday laboratory of clinical pathology. From the relatively small number of phase microscopes in use in clinical laboratories and the paucity of references to phase microscopy in the current American publications, it is suspected that this technique is experiencing the prolonged lag phase that so commonly intervenes between the discovery of a basic method and its adoption in the routine practice of medicine.

In all visual microscopy the recognition of an image depends solely upon differences in brightness (amplitude), in color (wave length), or in a combination of these two basic properties of transmitted light.[1] The conventional bright-field microscope is unable to render visible the differences in optical path (refractive index × thickness) inherent in an object. In the phase microscope, differences in optical path are translated into visible differences in light intensity (brightness, amplitude). If a specimen object contains differences in optical path or if an object has an index of refraction only slightly different from its surrounding medium, it can be visualized by phase microscopy. Only two alterations are necessary to change a bright-field microscope into a phase microscope. The condenser is replaced with a phase condenser which projects an annular band of light onto the object, and a phase plate (or diffraction plate), also of annular construction, is located at the back focal plane of the objective lens.

The theoretical foundation for the concept of phase microscopy was expressed by Ernest Abbe, the father of modern microscopy.[2] Around 1880, near the close of his period of scientific productivity, he submitted the theory that the image seen in the microscope is "the interference effect of a diffraction phenomenon." This concept, a revolutionary departure from the traditional interpretation of geometrical optics, was not accepted or understood by his contemporaries or successors for many years. F. Zernike,[2] of Groningen, is cred-

Submitted for publication June 6, 1957.

From the University of Southern California School of Medicine and the Santa Fe Hospital.

Chairman's address, read before the Section on Pathology and Physiology at the 106th Annual Meeting of the American Medical Association, New York, June 6, 1957.

ited with the discovery of phase-contrast microscopy, around 1930. In his Nobel Prize address, on the occasion of receiving the award in physics for 1953, Zernike described how his discovery grew out of his interest in the imperfections in diffraction gratings, first aroused in 1902 and the subject of his serious study from 1920 to 1930. The first phase-contrast microscopes were manufactured by Zeiss, and soon thereafter, around 1944, the first American phase microscopes were made by Spencer.[3]

The Application of Phase Microscopy

Phase microscopy is particularly suited to the examination of thin highly transparent objects in their native unstained state. The majority of microscopic objects of biological and medical interest belong to this group.[2] In traditional bright-field microscopy the structure of such objects is delineated by fixation and staining, which renders a color contrast within them. The early applications of phase were particularly in the field of biology, cytology, and bacteriology. Experience has shown that phase can be useful in the study of stained objects, and it has been combined with color contrast to advantage. It would be appropriate to generalize that phase microscopy can be of aid in delineating the structure of any transparent object, wherever a difference in optical path (refractive index × thickness) exists. Advanced techniques have adapted phase to fluorescent and polarization microscopy, infrared and ultraviolet light, and cinematophotomicrography, and it is now regularly used as an adjunct to electron microscopy.[4]

TABLE 1.—*The Language of Sixty-Three Titles in the "Current List of Medical Literature"* *

Language	No. Titles
German	23
English	17
Italian	9
French	8
Polish	2
Czechoslovakian	2
Spanish	1
Rumanian	1

* Reference 5.

TABLE 2.—*Topics of Phase Application Found in the Current List of Medical Literature* *

	No.
Bacteriology	12
Cytodiagnosis, tumors	11
Blood & bone marrow	9
Instrumentation	9
Histology, cytology	7
Neurohistology	2
Hematologic technique	4
Biology	3
Miscellaneous	6

* Reference 5.

My immediate interest has been in the simple phase microscopes that permit useful observations in the conventional clinical laboratory.

A review of the current publications indicates a much more popular application of phase in Europe than in the United States. Of the 63 titles on phase microscopy to be found in the "Current List of Medical Literature" [5] for 1956, 23 of the paper are in German; 9, in Italian, and 8, in French; of the 17 articles in English only a small minority were contributed by Americans (Table 1).

The current trends in the application of this method are reflected by the topics of the 1956 articles (Table 2). Most prominent are bacteriologic applications, the cytodiagnosis of tumors, the study of blood cells and bone marrow preparations, and histologic investigation and diagnosis.

Information on all aspects of phase microscopy, including the applications of phase in biology and medicine, up until 1950, has been conveniently collected by Bennett, Jupnik, Osterberg, and Richards.[1] Oscar W. Richards [6,7] published reviews on phase microscopy for the periods 1950-1954 and 1954-1956, accompanied by complete bibliography.

In the clinical laboratory there are many microscopic examinations which can be facilitated or enhanced by phase-contrast microscopic observation.[8,9] A list of the commoner applications of phase, of demonstrated practical value in clinical laboratory procedures, is as follows:

- Blood
  - Cell enumeration
  - Reticulocyte count
  - Platelet count
  - Differential white cell study
  - Leukemic cell identification
  - Supravital study
  - Bone marrow and spleen cytology
- Urine
  - Sediment, casts
- Vaginal exudate
  - Trichomonads
  - Fertility studies
  - Exfoliative cytology
- Sputum
  - Formed elements
- Semen
  - Abnormal sperm
- Feces
  - Ova
  - Parasites
- Microbiologic
  - Bacteria
    - Form and structure
    - Agglutination
  - (Viral inclusion bodies)
  - Fungi
    - Skin and hair
  - Parasites
    - Fecal ova, cysts, and adult forms
    - In body fluids and discharges
    - Malaria
- Histopathologic
  - Exfoliative cytology
  - Tumor cytology and fiber structure
  - Neuropathology
  - Tumor diagnosis

Phase has enjoyed a wide application to hematologic procedures.[10-12] The direct enumeration of platelets can be most accurately conducted with this method of observation.[13] There is an increasing use of phase in the identification of the various blood cells in leukemia.[14-16] Most often this is used as an adjunct to the study of stained smears, but there are those who believe that study of the unstained cells with phase is the most valuable single method for the identification of leukemic cells.[17] Plates illustrating the appearance of the various blood cells under phase microscopy have been published.[18-20] Phase microscopy is the natural complement to the technique of the supravital study of blood cells as introduced by Sabin.[21]

Phase microscopy is found to be far superior to bright-light microscopy in the various examinations for formed elements to be encountered in urine, vaginal discharge, feces, semen, sputum, and exudates.

There are bacteriologists who use phase as the standard microscopic method for the observation of bacteria. Spirilla are most easily found and identified by combined phase and dark-field technique, which is available in most instruments. Skin scales and hair are easily studied for fungus elements. Many parasites, in their various forms, are most easily encountered and identified with phase microscopy.[22]

There is a growing popularity in the use of phase microscopy in the examination of preparations for exfoliated cytologic elements.[23,24] The demands for cytologic diagnosis of suspected brain-tumor fragments early attracted the use of phase microscopy.[25] The application of this technique to the field of general tumor diagnosis has been furthered principally by workers on the Continent.[26,27]

Investigational Use

The use of phase microscopy in the rapid diagnosis of tumors by frozen section has been explored, with the aim of utilizing this method with conventional preparations. It is possible to prepare differentially stained rapid-frozen sections which render a completely acceptable histopathologic pattern readable with bright-light microscopy, but this ideal performance is not always realized in routine practice.

Frozen sections, cut on a standard freezing microtome, have been prepared by varying fixation, staining, and clearing. Fresh and hot and cold formalin-fixed tumor tissues have been used. The stained sections have been prepared with various simple water-soluble dyes commonly used for this purpose, especially the blue nuclear and oversight stains. Sections have been mounted in either water, glycerin, or 50% sugar or have been cleared through xylene and mounted in Permount. In routine sur-

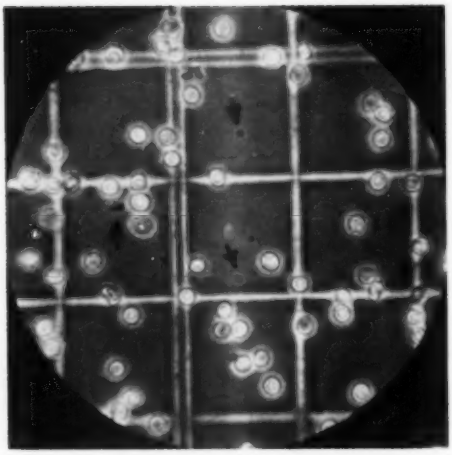

Figure 1

gical practice the first section of a specimen is mounted in glycerin and, while this is being studied, the second section is prepared by clearing through xylene. The Zeiss-Winkel Phase III Z/6 microscope has been used in the study of these preparations.

Thionine has been found to be a most useful stain for this purpose. As with other blue stains, the color rendition under phase is greatly altered, with an accentuation of the blue tinting in the ground substance and with certain cellular and nuclear structures, such as granules and nucleoli, showing up with a metachromatic deep red or purplish color. In contrast to its bright-light appearance, the coloration is quite spectacular. The use of special stains [28] and color-contrast phase [29] has not been explored. Although it is true that tissue patterns can be seen in unstained tissue, the addition of a nuclear dye has been of great aid to the traditionally trained pathologist.

Best and least confused detail has been seen in the sections cleared through xylene; glycerin clearing gave sections that were quite readable. The water and the saline mounts have been difficult to interpret because of the hyperdelineation of fibers and interfaces and confusing internal halation.

In summary of these observations several conclusions are drawn. Phase contrast can supplement bright-light microscopy by permitting the same surgical frozen tissue sections to be studied at a higher magnification without optical confusion and with higher resolution. It is most valuable when the routine preparation of a frozen section leaves much to be desired in the way of technical perfection, and occasionally it will salvage the diagnosability of an almost impossibly poor preparation. Such an optical advantage can be a boon to the surgical pathologist who is under pressure for a prompt and accurate diagnosis.

The accompanying illustrations are prepared to show the advantage of phase-contrast microscopy in situations in which the technical preparation is far from ideal.

Conclusions

Phase microscopy, which renders differences in refractive index visible as differences of amplitude (or light intensity), has

Figure 2

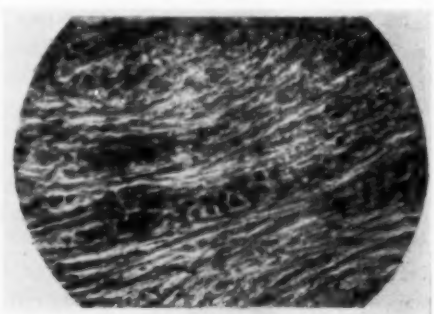

Figure 3

been found to be a valuable tool in the routine diagnostic methods in medicine as well as in the investigative fields of the basic biologic disciplines. It can be used in the routine micromorphologic methods of the laboratory of clinical pathology, particularly in the study of formed elements in discharges and exudates, in the counting of platelets, for the identification of leukemic blood cells, and in tumor-tissue diagnosis. It should, and undoubtedly will, enjoy wider application in the routine methods of the clinical laboratory.

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Carcinoma in Situ of the Stomach

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Carcinoma in situ has been discussed in the medical literature for the last decade probably more than any other single item in pathology and surgery. In 1908, Schottlander and Kermauner, and Schauenstein also in 1908, laid the basis for recognition of this entity, which was given its name by I. C. Rubin in 1918. Walter Schiller, in 1927 and years later, presented the most lucid description of this concept, crystallized the idea, and emphasized the lack of invasion of the basement membrane and the fact that carcinoma could be diagnosed without such invasion. Broders then popularized the entity.

Although most of the literature has dealt with carcinoma in situ of the cervix, it has been described in almost all the organs of the body, as in the skin, vulva, anus, mouth, pharynx, upper and lower respiratory system, urinary system,[7] gallbladder, cornea, conjunctiva, breast, etc. In the gastrointestinal tract it is not uncommon to find cellular changes at the periphery of an adenomatous polyp without invasion of the submucosa and thus, probably rightfully, diagnosed as carcinoma in situ. However, the true, nonpolypoid carcinoma in situ is extremely rare in the stomach. Most of the reports of carcinoma in situ in the stomach have been badly misplaced under the heading of "superficial spreading carcinoma," which is a clinical and pathological entity of its own.

It is the purpose of this paper to describe a true nonulcerative carcinoma in situ of the stomach, the diagnosis having been confirmed by several nationally prominent pathologists.

Submitted for publication April 26, 1957.

From the Departments of Pathology (Dr. Bocian) and Surgery (Dr. Geschke), Fresno Community Hospital.

A 53-year-old Armenian woman had complained for the previous two years of vague intermittent epigastric discomfort, usually in the form of a low substernal "burning," with occasional pyrosis unrelated to meals or degree of activity and recurring every four to five months, lasting about one week. There was no change in appetite, weight, or bowel habits; neither was nausea or vomiting present.

Her past history is generally noncontributory to her present clinical picture. Her father presumably died from cancer of the stomach at the age of 76. She underwent a cholecystectomy for a noncalculous gallbladder in 1931, while two years ago a subtotal thyroidectomy was done for an adenomatous goiter.

Physical examination disclosed a fairly well-developed and nourished patient in no discomfort at the time of survey. A blood pressure reading of 130/75 was obtained; her weight was 133 lb. Her various systems were found to be grossly normal. There was slight tenderness to deep palpation in the upper midepigastrium. Her blood picture was as follows: RBC, 3,800,000 (cells appearing normal); hemoglobin, 12 gm. per 100 cc.; WBC, 9200, with a differential count of 2 nonsegmented cells, 54 segmented cells, 1 basophil, 39 total lymphocytes, and 4 monocytes. The blood Wassermann test and the urinalysis were negative. Eight degrees of free HCl was obtained only after an hour following histamine injection.

A fluoroscopic and roentgenographic investigation of the upper gastrointestinal tract by Dr. C. L. Ould gave the impression of a change in outline along the distal portion of the greater curvature measuring 3.5 cm. in length and suggesting a moderate elevation of the mucosa which appeared fairly constant (Figs. 1, 2, and 3). Peristalsis was not unusual. At 60 minutes barium entered the small bowel, and at 3½ hours the stomach was almost empty and barium was in the ascending colon.

Despite the vague, almost trivial, symptoms and because of the questionable findings on x-ray examination, an exploratory laparotomy was decided on and carried out.

When the peritoneal cavity was entered, extremely dense and extensive adhesions were encountered, probably incident to the previous cholecystectomy. These had fixed the pyloroduodenal region to the under surface of the liver,

Fig. 1.—Evidence of slight pressure along the greater curvature in the distal stomach.

Fig. 2.—Disturbance in mucosal pattern with moderate elevation.

Fig. 3.—Evidence of pressure and mucosal elevation.

suggesting the possibility that this abnormal position might have been responsible for the change in outline on the roentgen film.

In the absence of any palpatory abnormalities of the area under suspicion, an exploratory gastrotomy was done. A longitudinal incision of about 7 to 8 cm. was made in the midportion of the antral region, permitting a good visualization as well as bidigital examination of the area in question. Again palpation did not yield any noticeable abnormalities. It was then thought that a biopsy specimen should be obtained from the location corresponding as nearly as possible to the portion designated as having "a change in outline" as reported by the radiologist. For this purpose the most prominent ruga was chosen, which, incidentally, appeared to be slightly pale but otherwise showed no variation from the neighboring

Fig. 4.—Low-power photomicrograph of lesion; reduced 15% from mag. × 15.

Fig. 5.—Atypical glandular cystic cavities, intestinalization, and increased branching and infolding. Hematoxylin and eosin; reduced 8% from mag. × 100.

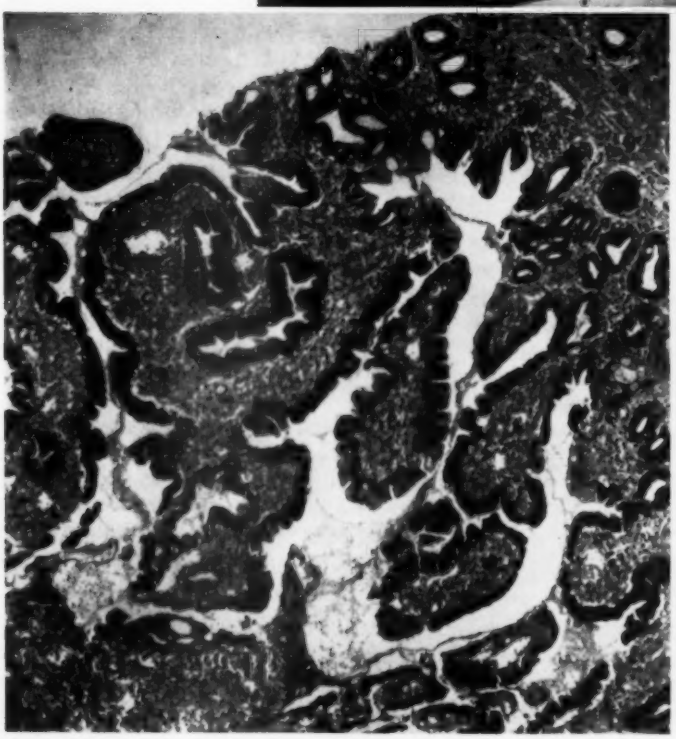

Fig. 6.—Higher-power view of lesion, showing hyperchromatic cells, many mitoses, and marked loss of polarity. Hematoxylin and eosin; reduced 15% from mag. × 400.

rugae. In fact, the primary motive for procuring a biopsy specimen was to have confirmatory evidence of the normal visual and palpatory gross findings on gastrotomy examination.

The gross specimen consisted of a formalin-fixed brownish soft circular but flat section of stomach wall 1.2×1.2×0.4 cm. The mucosa was grossly unaltered.

Microscopic findings consisted of markedly hyperplastic gastric mucosa to the point of its having intestinalized. It formed atypical glandular cystic cavities, some of which contained mucus, with increased branching and infolding. There were many mitoses, some abnormal. The cells lining the glands were hyperchromatic, showing a definite loss of polarity. Nuclei were different in size, shape, and staining qualities and were found at all levels of the cells. The transition between the normal and abnormal glands was quite abrupt. Multiple serial sectioning, step sectioning, and serial block examination failed to reveal any evidence of submucosal extension. A diagnosis of carcinoma in situ was thus made.

Because the surgeon could not be reasonably sure of the extent of the tumor or whether there might not be multiple tumor areas, a high subtotal gastrectomy was done, with the resection of five-sixths of the stomach, omentum, and subpyloric nodes. Multiple sections showed no further evidence of pathologic changes except for a chronic gastritis around the area of the previous biopsy. The patient has been in good health, and now, one and one-half years later, x-ray films show a well-functioning stoma of the anterior gastroenterotomy, with a normal mucosal pattern. Findings of the gastric analysis were similar to the one done before surgery.

Comment

The first and most-quoted report on carcinoma in situ of the stomach is that of Mallory,[11] in which he described and illustrated four such nonpolypoid lesions which, however, were already associated with peptic ulceration. The fact that our lesion failed to ulcerate might be explained on the basis of the almost complete achlorhydria. It is possible that the marked hypochlorhydria resulted from suppression even by this tiny carcinoma in situ.

Stout[13] classifies 200 cases of carcinoma of the stomach as follows, which is a slight modification of Barrman's classification:

I. Fungating, 25%; invades and metastizes late
II. Spreading; spreads along the stomach wall without the formation of a mass
  A. Superficial, 14%; spreads along the mucosa and submucosa
    1. Ulcerating, 80%
    2. Nonulcerating, 20%
  B. Linitis plastica, 2.5%; spreads along the submucosa, muscularis, and subserosa
III. Penetrating, 20%; grows through serosa and destroys muscle
IV. Unclassifiable, 32.5%

The so-called superficial spreading carcinoma is particularly under consideration in this article. Many authors and some of the latest textbooks have grouped under the same heading carcinoma in situ and superficial spreading carcinoma of the stomach and treat the two conditions interchangeably.[2] The fallacy of such a classification has been noted by Stout[13] and by Fennell and Castleman.[6]

The superficial spreading carcinoma is found mostly in the antrum and pylorus and remains superficial for a number of years. Radiologically it is characterized by a minimal ulceration, which might cover large areas, and by a large surrounding area in which peristalsis is absent. It grows along the mucosa and submucosa and obliterates the mucosal folds. By the time gastrectomy is performed 35% to 50% of cases have already metastasized to the regional lymph nodes. Some of these will show tumor emboli in the lymphatics of the mucosa even though the microscopic section fails to show submucosal involvement.

A carcinoma in situ, on the other hand, is characterized by the appearance of abnormal cells within the normal confines of the epithelial layer. These cells manifest

the appearance and behavior of cells found in malignant tumors, and although they may entirely replace the normal epithelium over large areas, the barrier of the basement membrane is never passed. It is obvious that superficial spreading carcinoma cannot be synonymous with the names "intraepithelial" and "noninvasive," as the synonyms of carcinoma in situ express. Moreover, the situation in the stomach should be similar to that of the cervix. If, after a diagnosis of carcinoma of the cervix is made, serial sections of more tissue reveal evidence of extension beyond the basement membrane, the original diagnosis of carcinoma in situ must be discarded. When we thus limit the diagnosis of carcinoma in situ of the stomach, it becomes an extremely rare finding.

The same fallacy has extended itself to other regions in the body, and an occasional pathologist will diagnose a carcinoma in situ of the endometrium from a curettage alone. How can such a diagnosis be made without having recourse to the underlying structures? This is like attempting to diagnose a carcinoma in situ of the cervix when only the peripheral portion of the cervical epithelium is included in the section.

In the same vein one might question the correctness of the title of the very interesting report by Foot and Papanicolaou,[7] "Early Renal Carcinoma in Situ. . . ." Have we come to the point where we can differentiate between "early" and "late" carcinoma in situ?

Summary

A case is reported of carcinoma in situ of the stomach first noted by the radiologist as a change in the outline on the greater curvature. The surgeon, after finding a normal gross mucosa, took a biopsy specimen from the place corresponding to the area of abnormality found in the x-ray.

A distinct separation must be made between the entities carcinoma in situ and superficial spreading carcinoma of the stomach. Other misused terms of the entity carcinoma in situ are discussed.

Conclusion

The radiologists quite often find certain abnormalities in the stomach wall in the form of abnormal peristalsis, a change in outline, disturbance in the mucosa, infiltrations, etc. The surgeon often reports negative findings and closes the stomach. It is possible that if biopsies were done on such stomachs, as in the case presented here, many early carcinomas might be detected. It is well known that carcinomas in situ in general quite often will present a normal gross appearance. Anything that will lower the rate of mortality from cancer of the stomach, which numbers 25,000 to 40,000 per year in the United States, is worth trying.

Dr. M. B. Dockerty, from Mayo Clinic, and Dr. William Meissner, from the Lahey Clinic, studied the slides, and Dr. S. F. Marshall, from the Lahey Clinic, studied the case.

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Primary Squamous-Cell Carcinoma of the Corpus Uteri

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Squamous epithelium in the endometrium has been described associated with malignant lesions of the corpus uteri. However, these lesions must meet certain rigid criteria, according to Fluhmann,[2] in order to be classified as primary squamous-cell carcinoma of the uterine body. These criteria are as follows: 1. There must be no sign of a coexisting cylindrical-cell carcinoma. 2. There must be no connection between the uterine cancer and the stratified squamous epithelium of the cervix. 3. The cervix must have been carefully scrutinized to rule out an original growth. Few bona fide reports meeting these criteria have appeared in the medical literature. The case herein reported meets these criteria.

Report of Case

A 59-year-old Negro woman, sextipara and sextigravida, was admitted to Lincoln Hospital for the first time on Nov. 16, 1953. Her chief complaint was a foul brown vaginal discharge of three to four weeks' duration accompanied by severe abdominal cramps for one week prior to admission. The menopause had occurred more than 10 years previously, with no history of vaginal bleeding or discharge until the present history. There was also a history of diabetes mellitus of three years' duration, for which 20 units of protamine zinc insulin injection was given daily. The pertinent physical findings were an irregularly enlarged uterus the size of a 10-week gestation. The uterus was moderately tender to palpation. The adnexae were of normal size and freely movable. Speculum examination revealed no abnormality of vagina or external os other than a brown discharge coming from the cervix.

The patient was operated on nine days after admission. A total hysterectomy and bilateral salpingo-oopherectomy was performed. At operation an enlarged soft symmetrical uterus was found, and the surgeon interpreted the gross lesion as "carcinoma of the corpus." The adnexae were normal. No evidence of metastatic lesion was noted. Three weeks after admission the patient was discharged.

In April, 1954, five months after operation, there was noted extensive recurrent disease in the vaginal vault, with parametrial involvement. A biopsy of the vaginal vault was done. High-voltage x-ray for palliation was begun, but the patient died suddenly two months later (seven months after operation). There was no post-mortem examination.

Pathological Examination of Surgical Specimen

Gross Examination

The specimen consisted of uterus, cervix, both Fallopian tubes, and both ovaries. The uterus was symmetrically enlarged and measured 9.6×6.4×4.5 cm. The cervix measured 3×2 cm. The cervical canal was patent and lined by smooth gray-yellow mucosa raised into longitudinal folds. There was no area of tumor, ulcer, or scar formation. The endometrium could not be identified, as the cavity was full of a fungating pale red necrotic tissue attached to the myometrium on all surfaces. The myometrium was infiltrated by this soft tumor tissue and was of varying width, measuring up to 1.5 cm. This mass filled the entire uterine cavity, except for a small portion 1 cm. above the internal os. In this latter area the endometrium was granular, showing small papillary-like elevations. The consistency of the tumor mass was soft in the necrotic areas, but other areas were nodular and more firm to palpation. Both Fallopian tubes showed essentially a similar normal architecture. They measured 7 cm. in length and 0.5 to 0.8 cm. in diameter. The lumens were patent, and the mucosa appeared normal. Both ovaries measured 3.5×2.0×1.5 cm. Their consistency was firm, and the cut surface showed no gross evidence of neoplasm.

Microscopic Examination

To fulfill the criteria as outlined above, serial longitudinal sections of the cervix and endocervical canal were done. These

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From the Departments of Pathology and Gynecology, Lincoln Hospital.

Fig. 1.—External os of cervix, showing normal squamous epithelium; hematoxylin and eosin stain.

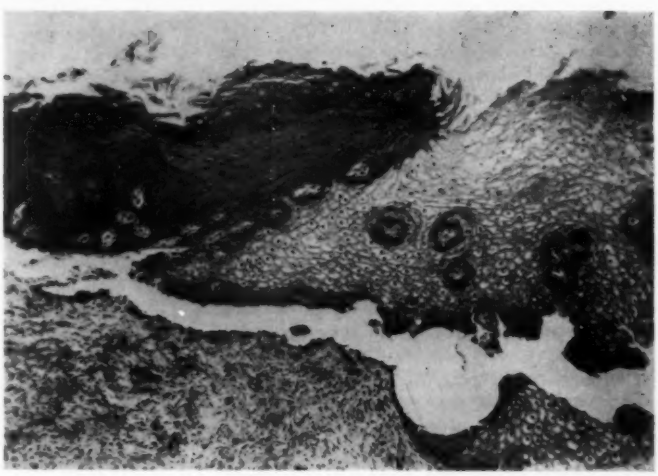

Fig. 2.—Squamocolumnar junction and endocervical canal. Squamous epithelium replaces normal mucosa; hematoxylin and eosin stain.

serial sections were taken from the external os to a distance of 1.8 cm. above the squamocolumnar junction. Above this level, sections in the long axis of the endocervical canal to include the mucosa and entire thickness of the cervix were taken. Sections of the endometrium 1 cm. above the internal os, as well as multiple sections of the

Fig. 3.—Endocervical glands, showing epidermization; hematoxylin and eosin stain.

fungating tumor from all surfaces, were also taken.

The following were the pertinent histological findings: 1. External os of cervix showed normal squamous epithelium. The underlying stroma showed no unusual changes (Fig. 1). 2. Sections at the squamocolumnar junction and endocervical canal revealed mucosa replaced by squamous epithelium throughout its length (Fig. 2). Various stages of epidermization not only at the surface but also in a few of the glands were seen (Fig. 3). No evidence of invasive or interepithelial malignancies was noted. 3. Sections of the endocervical canal revealed squamous epithelium replacing columnar mucosa. The entire thickness of the wall showed no evidence of malignant cells. 4. Endometrium immediately above the internal os revealed squamous epithelium replacing the endometrial mucosa (Fig. 4). 5. Sections of the fungating tumor mass on all surfaces revealed identical histological structure, namely, squamous-cell carcinoma with squamous and keratinized differentiation and formation of "pearls" (Fig. 5). Invasion into the myometrium by tumor was also noted. 6. Biopsy of vaginal vault metastasis removed five months after operation revealed well-differ-

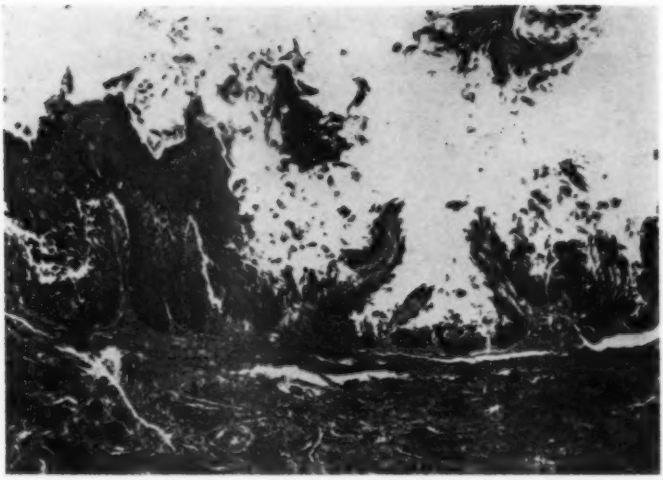

Fig. 4.—Endometrium immediately above internal os. Squamous epithelium replaces endometrial mucosa; hematoxylin and eosin stain.

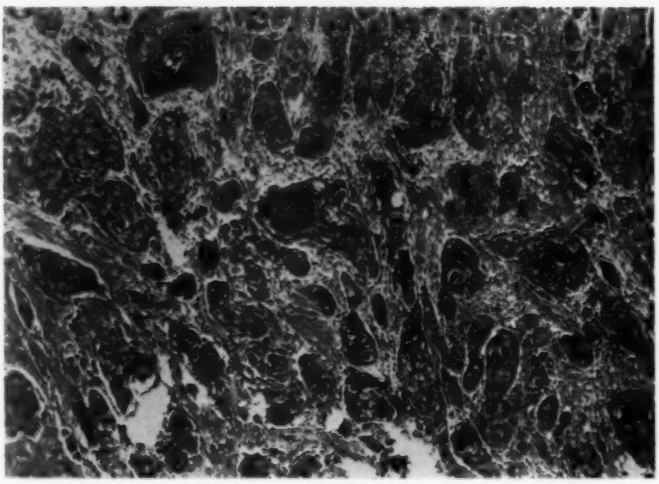

Fig. 5.—Fungating tumor mass, showing "pearls" and invasive features; hematoxylin and eosin stain.

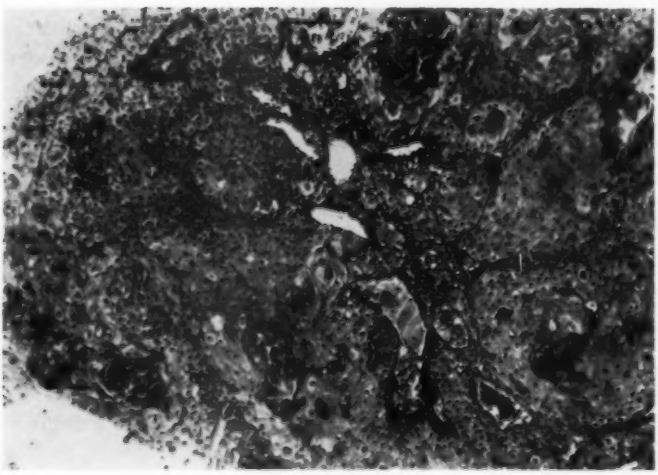

Fig. 6.—Vaginal-vault biopsy specimen, showing squamous-cell carcinoma metastases; hematoxylin and eosin stain.

entiated metastatic squamous-cell carcinoma (Fig. 6).

Comment

Prior to 1928, Fluhmann [2] collected two cases from the literature as being bona fide primary squamous carcinoma of the corpus uteri and meeting the criteria as mentioned above. Three other cases reported prior to that date were mentioned but not accepted by Fluhmann because of incomplete or no examination of the cervix. In our case this difficulty was adequately overcome. Review of the literature since 1928 has revealed an additional case, reported by Wahl and Jain.[5]

The finding of squamous cells in the uterine body has been noted very frequently in benign diseases, in malignant diseases, and in fetal and early childhood development.[4] It has been described in endometritis, by Zeller [7]; in specific types of endometritis, by Alterthum,[1] and after pregnancy, by Hengge.[3] Wilson and DuBois [6] described similar findings in a 5-month-old child who died of vitamin A deficiency.

Squamous-cell epithelium had been found in the corpus uteri as a metaplastic process in association with adenocarcinoma of the uterus, or coexisting as squamous-cell carcinoma with an adenocarcinoma, or secondary to a similar growth in the cervix. As a primary carcinoma of the uterine body meeting the rigid criteria described above, it has been reported only on three different occasions.

Our case is interesting in that it does not manifest on microscopy any element of glandular carcinoma. There is also no evidence of chronic endometritis. There is marked metaplasia of the endocervical canal and in the endometrium for 1 cm. above the internal os. This would lead us to suspect that primary squamous-cell carcinoma arose from the squamous metaplasia of the endometrium. We can only speculate as to the etiology of this metaplasia.

Summary

A case of primary squamous-cell carcinoma of the corpus uteri, with local metastasis and showing squamous metaplasia of the endometrium and endocervical canal, is reported. The rigid criteria for this diagnosis are noted and fulfilled.

Dr. Arthur Hertig reviewed the slides in this case.

E. 141st St. & Concord Ave. (54).

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Pathogenesis of Serum Sickness

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For both the morphologist and the immunologist, experimental serum sickness has been the most promising laboratory model for the study of a variety of human diseases of presumed hypersensitive etiology. The convincing demonstration by Rich and Gregory of the production of serum sickness in rabbits with lesions resembling those of periarteritis nodosa,[1] rheumatic fever,[2-4] disseminated lupus erythematosus,[4,5] rheumatoid arthritis,[5] and glomerulonephritis[6] initiated studies which have made experimental serum sickness one of the most thoroughly investigated of hypersensitive states. The early work of Longcope and Rackemann[7] and Mackenzie and Leake,[8] the later observations of Rich and Gregory,[1-6] Hopps and Wissler,[9] and Ehrich and associates,[10] and especially the studies of Hawn and Janeway[11] and Germuth,[12,13] employing purified antigens, have established beyond much doubt that the morphologic lesions of serum sickness are the result of an antigen-antibody reaction. It has been repeatedly demonstrated that this reaction occurs as the host begins to form antibody while antigen is still persisting in his blood and tissues and that with the continued production of antibody antigen is eliminated, free antibody appears in the circulation, and the manifestations of serum sickness disappear.

Beyond these points of general accord, however, little has been learned concerning the mechanisms by which the antigen-antibody reaction produces the characteristic lesions of this disease. A number of possible mechanisms have been suggested. First, antigen might become localized in the sites of the lesions prior to the formation of antibody, and with the subsequent appearance of antibody in these sites the resultant antigen-antibody reaction could cause the characteristic tissue changes.[8,11,14] Second, antibody might become fixed in certain sites and then react with circulating antigen to produce the lesions.[10] Third, there might be no fixation of either antigen or antibody prior to their reaction,[6] and antibody might in this event reach the sites of specific lesions in the form of soluble antigen-antibody complexes.[12] Fourth, the

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Department of Pathology, University of Pittsburgh School of Medicine; Public Health Service Research Fellow of the National Institute of Neurological Diseases and Blindness (Dr. Weigle); Sarah Mellon Scaife Fellow in Pathology (Mr. Cochrane).

Presentation of Parke-Davis Award

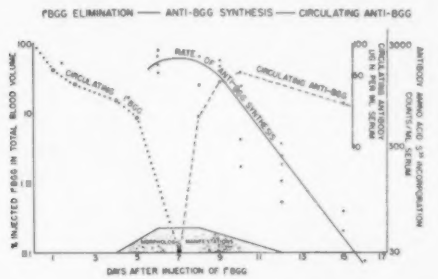
FBGG ELIMINATION — ANTI-BGG SYNTHESIS — CIRCULATING ANTI-BGG
% INJECTED FBGG IN TOTAL BLOOD VOLUME
CIRCULATING FBGG
RATE OF ANTI-BGG SYNTHESIS
CIRCULATING ANTI-BGG
CIRCULATING ANTIBODY UNITS IN PER ML SERUM
ANTIBODY AMINO ACID S35 INCORPORATION COUNTS/ML SERUM
IMMUNOLOGIC MARROW CHANGES
DAYS AFTER INJECTION OF FBGG

Fig. 1.—Immunologic and morphologic events transpiring after primary injection of I*BGG into rabbits.

antigen-antibody reaction, without being localized in the sites of tissue damage, might produce focal tissue injury via toxic products or vascular spasm.[9] Now, in the light of recent observations, it may be possible to resolve some of these conflicting ideas and come a step or two closer to the understanding of the pathogenesis of serum sickness.

To begin with, let us review briefly the sequence of immunologic and morphologic events occurring subsequent to the injection of a large dose of serum protein antigen in rabbits. Figure 1 illustrates the happenings after the intravenous injection of I^{131}-labeled bovine γ-globulin (I*BGG).[15] The familiar three-phase curve of antigen elimination from the blood is shown by the dotted line at the left and reflects (1) an equilibration of the antigen between intravascular and extravascular components of the body fluid during the first two days, (2) a two-day period of relatively slow nonimmune catabolism of I*BGG prior to the formation of antibody, and (3) a final rapid elimination of I*BGG resulting from the formation of anti-BGG. In considering the fate of antigen in the tissues of the rabbit it should be pointed out that the levels of I*BGG in the various tissues tend to parallel closely the levels of circulating I*BGG.[16] As you are aware, results of tracing serum protein antigens with the I^{131} label indicate complete removal of antigen from the circulation prior to the appearance of free precipitating antibody and virtually complete elimination of antigen from the tissues shortly thereafter. In contrast to these observations, several groups of workers have injected similar antigens into rabbits and using other techniques have found antigen-like materials in the blood and tissues for long periods of time.[17-19] In some respects these antigen-like materials, the precise nature of which is still undetermined, were physically and chemically different from the original antigen, but they did have a similar immunologic specificity. However, this controversy regarding persistence of antigens or antigen-like materials need not concern us here, since, whatever the nature of these persisting materials, they do not seem to play a discernible role in the course of antibody production or in the development of serum sickness.

After the elimination of circulating I*BGG, the subsequent rise and fall in circulating precipitating anti-BGG are indicated by the dashed line in Figure 1. Free anti-BGG is detectable as soon as I*BGG is eliminated from the circulation, and the maximum concentration of circulating anti-BGG is found three days after elimination of I*BGG. After reaching maximum concentration in the blood, the concentration of anti-BGG falls at a rate close to the half-life of passively transferred antibody, indicating little further anti-BGG synthesis.[16] The solid curve beginning on the fifth day after injection of BGG indicates the actual rate of anti-BGG synthesis as measured by the uptake into anti-BGG of S^{35}-labeled amino acids injected into different animals at various times during their response.[20] Rates of antibody synthesis during the first few days of response are low, and precise measurements are difficult to obtain. However, it is apparent that the rate of anti-BGG synthesis increases rapidly while circulating BGG is present, and the maximum rate of synthesis is reached about the time the circulating BGG is exhausted. Subsequently, anti-BGG synthesis decreases at a fairly constant rate. The period of development of morphologic

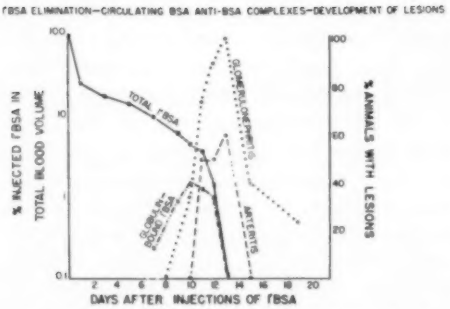

Fig. 2.—I*BSA-induced serum sickness in the rabbit. Immunologic and morphologic events transpiring after the primary injection of I*BSA into rabbits.

manifestation of serum sickness in this situation is indicated by the shaded area below. These morphologic manifestations include a proliferative glomerulonephritis, arteritis, and endocarditis including valvulitis. As has been pointed out previously, the lesions appear while antigen is being rapidly eliminated and they disappear after the appearance of circulating antibody. Now another correlation can be made, i. e., appearance and disappearance of the serum-sickness lesions closely parallel the rise and fall of antibody synthesis.

More detailed information concerning the interaction of antigen and antibody during the development of serum sickness is obtainable when crystalline bovine serum albumin (BSA) is employed as the antigen. Figure 2 illustrates some of the events following the injection into rabbits of 0.25 gm. of I^{131}-labeled BSA (I*BSA) per kilogram, according to the method of Germuth.[12] The typical pattern of elimination of circulating I*BSA shown by the solid line at the left has the same three phases as seen in Figure 1 for I*BGG, but the nonimmune elimination phase is somewhat longer. Because the rapid elimination of antigen occurs at the time of antibody synthesis, it is logical to assume that the antibody as it is formed combines with antigen in soluble complexes, since the blood and tissues are in a state of antigen excess, and then the complexes are removed from the circulation. Throughout the response to an injection of I*BSA it is possible to determine the amount of circulating I*BSA which might be combined with antibody in the form of soluble antigen-antibody complexes. To do this we have employed the ammonium-sulfate-precipitation technique of Farr.[21] In this procedure the serum containing I*BSA is fractionated at 50% ammonium sulfate saturation, which precipitates the serum globulin plus any I*BSA combined with globulin (i. e., antibody) and leaves free I*BSA in the supernatant. After this separation isotopic measurement of globulin-bound I*BSA and free I*BSA is made.

The dot-dash line in Figure 2 indicates the percentage of total I*BSA injected which was found bound to globulin in a typical rabbit. From the base line values found up to the 7th day after injection the amount of I*BSA in complex form increased sharply to maximum values on the 10th day and then decreased, paralleling the fall in total circulating I*BSA. During the rapid elimination of I*BSA as much as one-half to three-fourths of the circulating I*BSA was bound to the host's globulin. Occasional rabbits will, however, form an antibody capable of combining with antigen without increasing its rate of elimination from the blood. In this situation antigen circulates in the form of antigen-antibody complexes and the antibody response cannot be detected by the usual tests for antigen or antibody. In 3 such rabbits serum complement levels were unchanged while the antigen-antibody complexes were present in the circulation, in contrast to the usual drop in complement associated with the formation of complexes which are rapidly eliminated from the blood. In two of these rabbits serum-sickness glomerulonephritis was observed during the course of what appeared to be nonimmune antigen elimination. In x-rayed rabbits given injections of similar amounts of I*BSA no antibody response occurred and no I*BSA host globulin complexes were demonstrable.

Also in Figure 2 is recorded the percentage of animals killed at various intervals which displayed the glomerulonephritis (dash line) and arteritis (dot line) of serum sickness. The incidence of glomerulonephritis increased during the immune elimination of antigen to 100% at the time of complete antigen elimination and then promptly declined. During the same period the incidence of rabbits with arteritis rose to 60% and rapidly declined. These incidences are quite similar to those reported by Germuth.[12] From these observations it is apparent, as has been suggested by others, that the development of serum sickness lesions occurred at the time of antigen-antibody interaction. In addition, we can now add that the lesions developed while a considerable amount of antigen-antibody complex was present in the circulation.

Studies of the properties of such antigen-antibody complexes formed in antigen excess indicate that they have considerable biological activity. Soluble BSA-anti-BSA complexes formed in antigen excess in vitro will induce anaphylaxis in normal guinea pigs, cause intense local inflammatory reactions following subcutaneous injection into normal rabbits, and produce serum sickness-like glomerulonephritis following intravenous injection in normal rabbits.[22,23] Thus it seems entirely possible that complexes formed during the host's immune response might play a key role in the development of the lesions of serum sickness.

The next phase of these studies dealt with the immunohistochemical analysis of the tissues of rabbits receiving large doses of BSA. Particular emphasis was placed on analysis of the serum sickness lesions for BSA and anti-BSA and also for the host's γ-globulin (RGG) and albumin (RSA). This work was done with use of the fluorescent antibody technique of Coons and Kaplan.[24] The hypothesis that in serum sickness antigen is first fixed in certain tissue sites and then later antibody reaches these sites and the ensuing antigen-antibody reaction causes the morphological lesions was tested. Glomeruli, arteries, and hearts of rabbits receiving BSA were searched for concentrations of antigen during the interval between the injection of antigen and the onset of antibody formation or serum sickness. All these studies were negative. In addition, rabbits were x-rayed to negate their immune response and then given injections of the usual serum-sickness-producing dose of BSA. These animals showed no antibody response or serum-sickness lesions, and their glomeruli, arteries, and hearts showed no accumulations of BSA

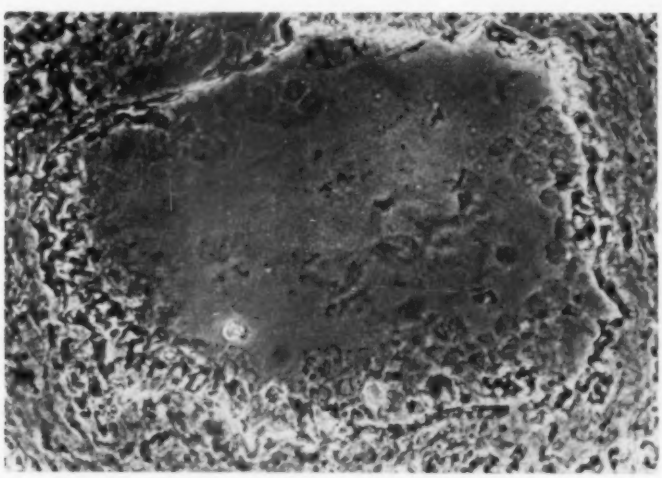

Fig. 3.—Phase-contrast photomicrograph of frozen section of medium-sized coronary artery, showing typical serum-sickness arteritis following injection of BSA. Internal elastic lamina appears as bright wavy refractile line about lumen. Intimal proliferation is evident in lower left third of circumference of the vessel. In this section the regular laminated structure of the arterial wall is also altered by inflammatory reaction.

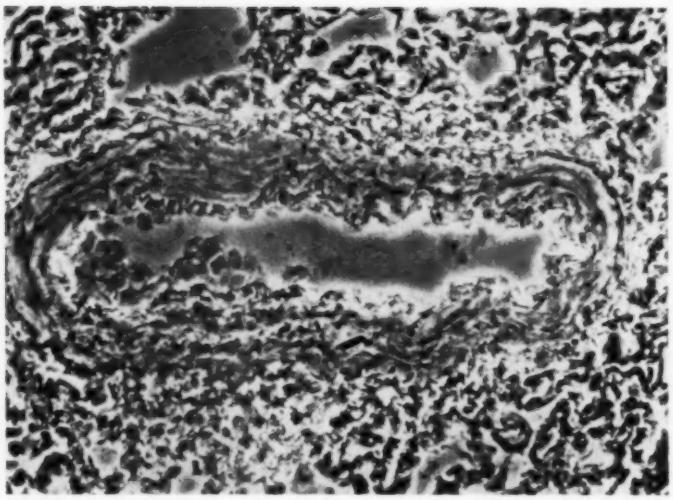

Fig. 4.—Phase-contrast photomicrograph of frozen section of small coronary artery, showing focal arteritic lesion following injection of BSA. In the lower left aspect of this vessel a proliferation of the intima and distortion of the media with fragmentation of elastic fibers is evident.

11 days after injection, at a time when nonirradiated rabbits showed a near maximum incidence of lesions of serum sickness in these sites. Thus, within the limitations of this technique, there was no demonstrable fixation or localization of antigen in sites where serum sickness lesions usually occur either prior to the development of such lesions in animals presumably destined to get serum sickness or in x-rayed animals not responding to the antigen.

Next, active lesions of serum sickness were analyzed for the offending antigen, BSA. In all active arteritic lesions and in the glomeruli of the kidney showing glomerulonephritis there was a readily demonstrable concentration of BSA. The involved arteries showed characteristic intimal proliferation, edema, infiltration of inflammatory cells, and development of fibrinoid (Figs. 3 to 5). In these lesions there was a coarse irregular granular deposition of BSA most marked at the level of and just outside of the internal elastic lamina (Figs. 6 to 8). This deposition of BSA was evident in areas of arteritis, but it was not uniform throughout the entirety of these lesions. In sections showing inflammatory involvement of only part of the circumference of an artery, BSA was demonstrable only in the involved area and not in the segment of normal arterial wall. There was no BSA demonstrable in histologically normal coronary, renal, or hepatic arteries. However, in occasional splenic arterioles of some of the rabbits with active serum sickness small amounts of BSA were detected without histologic evidence of associated damage (Figs. 9 and 10). Why this occurred in the splenic vessels and not elsewhere is not apparent. The glomeruli in the rabbits with serum sickness showed typical proliferative changes (Figs. 11 and 12). In these involved glomeruli the BSA appeared as a fine granular deposit, at times in an arrangement suggesting the outline of walls of the glomerular capillaries (Fig. 13). Again, glomeruli in kidneys not showing glomerulonephritis contained no demonstrable BSA. Since no decision could be reached as to whether deposition of antigen preceded morphologic alteration or vice versa, it seems that the two processes must proceed almost simultaneously. Likewise, with the prompt regression of the inflammatory lesions after the elimination of antigen from the circulation the BSA in these sites also disappeared. No BSA was demonstrable in vessels or glomeruli of rabbits which had recovered from serum sickness.

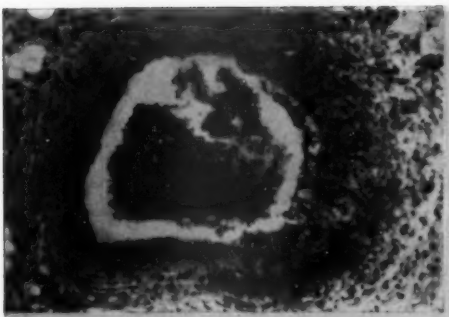

Fig. 5.—Photomicrograph of hematoxylin-and-eosin-stained paraffin section of coronary artery with arteritis after the injection of BSA, similar to that seen in Figure 3. Intimal proliferation and inflammatory infiltration of media are evident at right, while fibrinoid change in media and adventitia can be seen at lower right.

Fig. 6.—Fluorescence photomicrograph of identical field seen in Figure 3 and similar to that seen in Figure 5, stained with fluorescent anti-BSA to identify responsible antigen. The internal elastic lamina is evident as wavy blue line about lumen, best seen in unaffected sector of vessel at upper right. Light-green fluorescent material seen in wall of the artery at bottom and left indicates presence of BSA in area of arteritis. The BSA appears to be at the level of the internal elastic lamina and throughout the muscular media. The deposition of BSA corresponds to the region of morphologic alteration of the vessel seen in Figure 3.

Fig. 7.—Fluorescence photomicrograph of identical field seen in Figure 4, stained with fluorescent anti-BSA to identify responsible antigen. The blue autofluorescence of the tissue due primarily to elastic fibers outlines the anatomy of the artery. In the site of the arteritic lesion seen at left lower aspect of vessel in Figure 4 is a light green fluorescence, indicating depositon of BSA within the lesion. Again, BSA appears to be primarily within the media near the internal elastic lamina.

Fig. 8.—Fluorescence photomicrograph stained with fluorescent anti-BSA of frozen section of coronary artery, showing arteritis after injection of BSA. Artery is cut longitudinally, with lumen at right. Internal elastic lamina appears as distinct blue autofluorescent line at right, and the elastic fibers of the adventitia are seen as less definite blue-staining band to left. Foci of bright-green fluorescence in media adjacent to internal elastic lamina indicate presence of BSA.

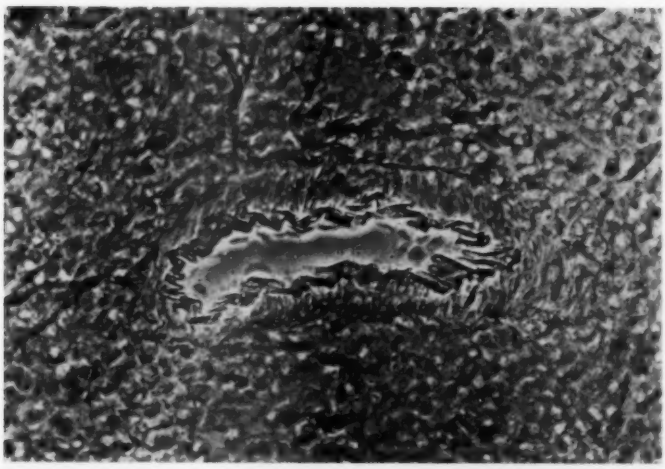

Fig. 9.—Phase-contrast photomicrograph of frozen section of splenic arteriole from rabbit exhibiting glomerulonephritis and coronary arteritis following injection of BSA. This arteriole shows no morphologic abnormality.

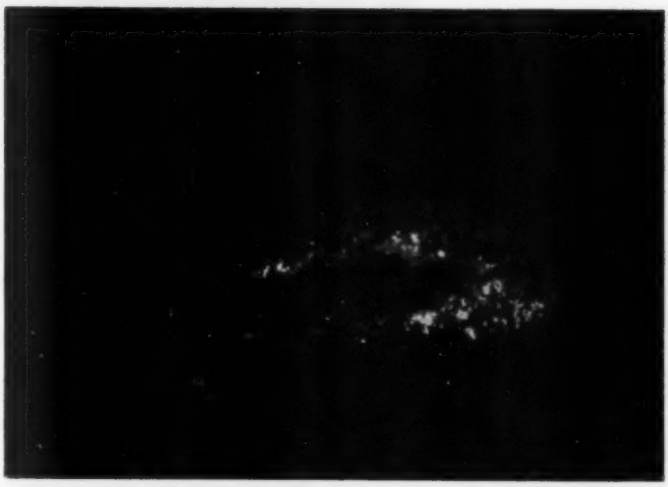

Fig. 10.—Fluorescence photomicrograph of identical field seen in Figure 9, stained with fluorescent anti-BSA to identify injected antigen. Note fine granular fluorescence at right, indicating presence of BSA not associated with morphologic alteration.

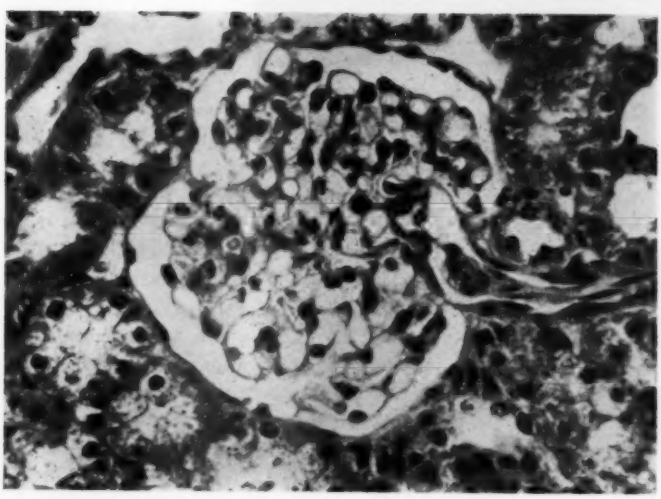

Fig. 11.—Photomicrograph of hematoxylin-and-eosin-stained paraffin section of normal rabbit glomerulus.

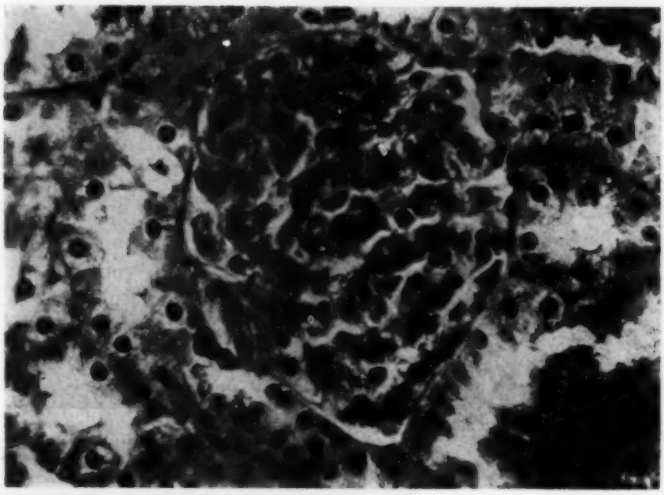

Fig. 12. — Photomicrograph of hematoxylin-and-eosin-stained paraffin section of glomerulus of rabbit given injection of BSA two weeks previously. Typical proliferation of epithelial and endothelial cells is evident. Tubules appear essentially normal.

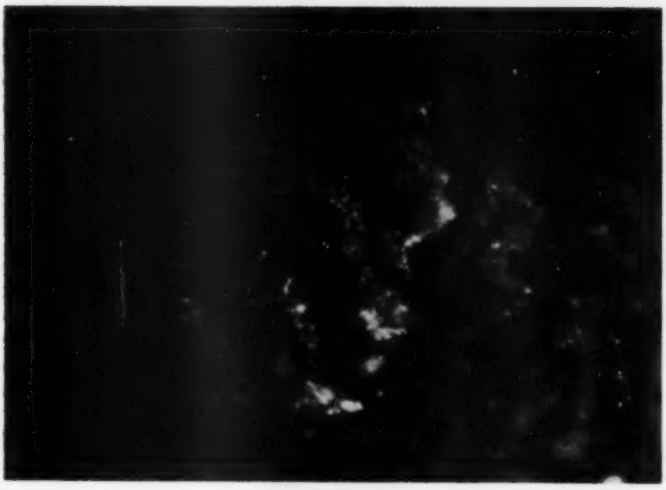

Fig. 13.—Fluorescence photomicrograph of frozen section of glomerulus from rabbit, demonstrating glomerulonephritis following injection of BSA stained with fluorescent anti-BSA. Glomerulus occupies central two-thirds of Figure. Fine granular fluorescence appearing in part to exhibit a capillary-like pattern indicates presence of BSA in the glomerulus.

This demonstration of BSA in the serum-sickness lesion could not in itself be interpreted as evidence for a specific localization of antigen in the lesions, since it might be that any circulating protein would leak into and be retained in such inflammatory or degenerative lesions. In order to get some idea as to the specificity of this localization of BSA, animals in the course of developing serum sickness, approximately nine days after injection of BSA, were given an injection of human γ-globulin (HGG), 50 mg. per kilogram, an amount approximately equal to the amount of BSA remaining in their body, and were killed two days later, before they had made any detectable antibody response to HGG. The lesions of arteritis and glomerulonephritis in these animals were then examined for both BSA and HGG. In all instances the lesions contained appreciable concentrations of BSA but no localization or concentration of HGG. This observation would seem to indicate that the concentration of BSA in the serum-sickness lesions was specific and not a nonspecific outpouring and trapping of

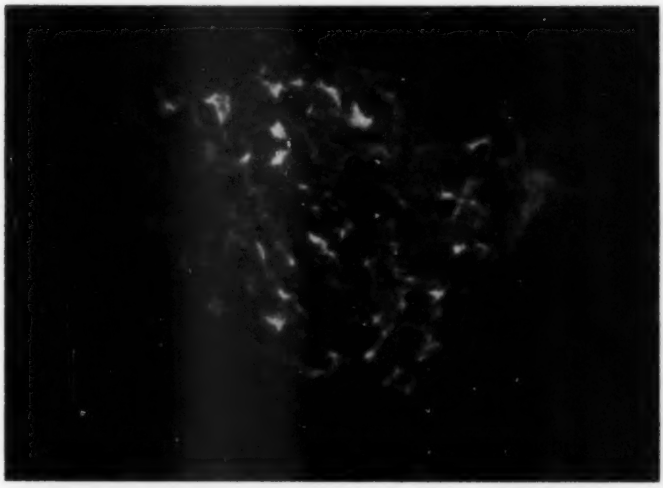

Fig. 14.—Fluorescence photomicrograph of frozen section of normal rabbit glomerulus stained with fluorescent anti-RGG. A similar distribution of RSA is seen in normal rabbit glomeruli.

foreign protein in areas of inflammation.

The arteritic and glomerulonephritic lesions were also studied for anti-BSA, which might be expected to be associated with the concentrations of BSA especially if deposition of antigen-antibody complexes was occurring. The presence of considerable amounts of BSA in these lesions complicated the evaluation of results obtained with the usual sandwich technique for detection of anti-BSA, i. e., addition of BSA followed by fluorescent anti-BSA.[25] However, the presence of anti-BSA could be inferred by a blocking technique. In this procedure sections of lesions stained directly with fluorescent anti-RGG were compared with adjacent sections which were first covered with BSA and then stained with fluorescent anti-RGG. The BSA specifically reduced the subsequent staining of the lesions with fluorescent anti-RGG, presumably by combining with anti-BSA and then partially blocking the RGG-fluorescent anti-RGG reaction.

Staining of the arteritic and glomerulonephritic lesions for RGG and RSA showed both these proteins to be present in increased amounts. As seen in Figures 14

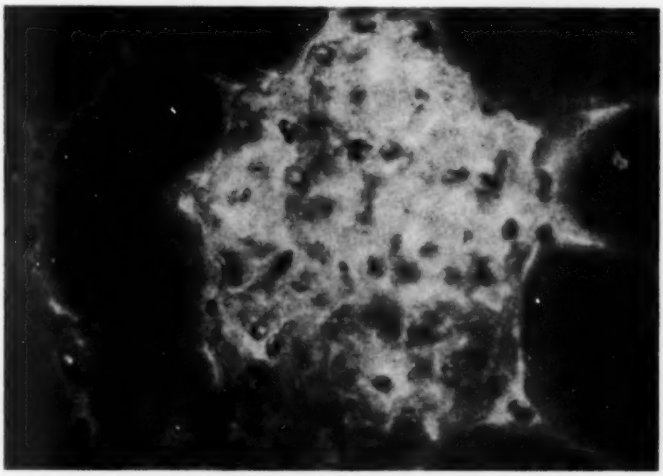

Fig. 15.—Fluorescence photomicrograph of frozen section of rabbit glomerulus, showing glomerulonephritic changes of serum sickness, stained with fluorescent anti-RGG. Exposure comparable to that for Figure 14. Note increased concentration of host's γ-globulin in diseased glomerulus.

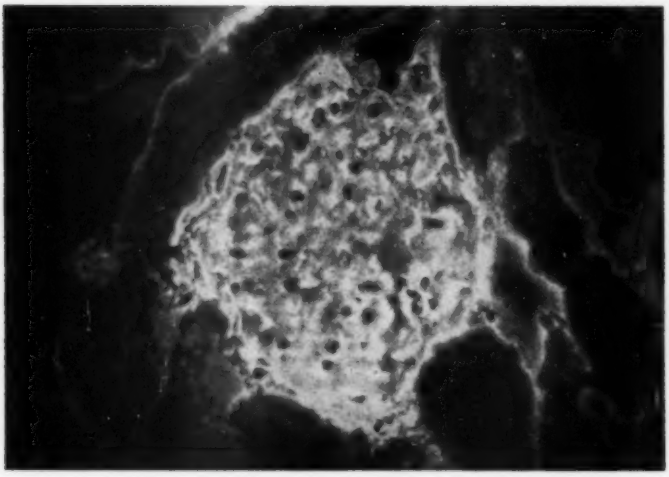

Fig. 16.—Fluorescence photomicrograph of frozen section of rabbit glomerulus, showing glomerulonephritic changes of serum sickness, stained with fluorescent anti-RSA. Exposure comparable to that for Figures 14 and 15. Note increased concentration of host's albumin in diseased glomerulus.

to 16, glomeruli showing the proliferative inflammatory lesions of serum sickness contained much more of the host's albumin and γ-globulin than did glomeruli of normal kidneys or normal-appearing glomeruli in serum-sickness animals. In addition, both these host's proteins were distributed much more widely in the lesions than the specific antigen, as shown in Figures 17 and 18. These observations suggest that the host's albumin and much of the host's γ-globulin found in such lesions may accumulate there nonspecifically as a part of the inflammatory exudate and point up the danger of attaching immunologic significance to the isolated demonstration of γ-globulin in tissue lesions.

Summary and Conclusions

On the basis of the present observations it appears the initiation and development of the morphologic manifestations of serum sickness (1) closely parallel the increase in rate of antibody formation; (2) occur while antigen-antibody complexes presumably capable of inducing inflammatory and anaphylactic responses are present in the circulation; (3) are accompanied by an almost simultaneous localization of antigen and probably antibody in the sites of the lesions. Also there is no detectable localization or fixation of antigen in those tissues predisposed to the development of lesions prior to the formation of antibody.

The regression of the lesions of serum sickness on the other hand (1) follows the disappearance of readily detectable circulating antigen and antigen-antibody complexes; (2) parallels a decline in rate of antibody production; (3) is accompanied by the disappearance of antigen from sites of the lesions.

With these facts at hand it is tempting to propose a mechanism by which the interaction of antigen and antibody might cause the development of the lesions of serum sickness. The sequence of events might run as follows. 1. As antibody begins to be formed it combines with antigen in an antigen-excess environment and forms soluble complexes which increase in amount with the increase in antibody synthesis until all antigen is eliminated. 2. These complexes circulating in the body fluids are presumably capable of initiating anaphylactic and inflammatory responses in the host. 3. Then by means not yet known these complexes initiate and are localized in allergic inflammatory lesions in certain arteries and glomeruli. Whether these lesions are initiated by stimuli derived from the circulating complexes and the concentration

Fig. 17.—Fluorescence photomicrograph stained with fluorescent anti-RGG of frozen section of coronary artery, showing arteritis following injection of BSA. Fluorescence indicates accumulation of host's γ-globulin throughout entire thickness of wall of vessel in the region of the lesion (lower right). Fluorescence within lumen indicates presence of RGG in the blood.

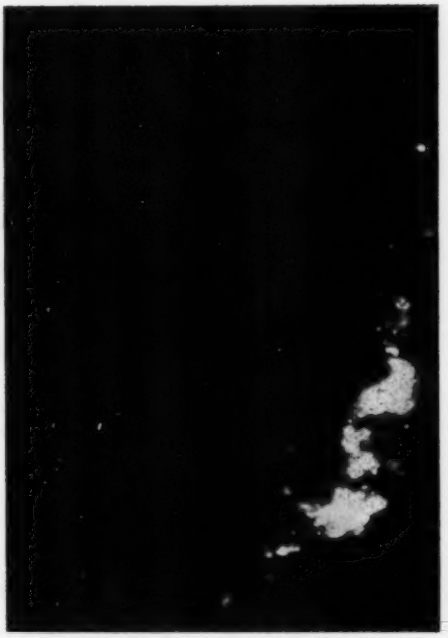

Fig. 18.—Fluorescence photomicrograph of same vessel as seen in Figure 17, same magnification and orientation adjacent section, stained with fluorescent anti-BSA to indicate distribution of offending antigen. The distribution of the antigen is more restricted than that of the host's own γ-globulin as seen in Figure 17. If antigen and antibody are deposited together in the lesions one might expect a similar distribution for both. This difference suggests that much of the host's γ-globulin present in the lesions may not be antibody localized by virtue of immunologic reaction but rather may be merely part of the nonspecific inflammatory exudate.

of the complexes in these sites is a secondary phenomenon of phagocytosis or whether the deposition of complexes in vessel walls provides the initial inflammatory stimulus remains to be determined. The precise position of the lesions might depend upon local physiological conditions at the time the complexes first appear. 4. The development of the inflammatory lesions and localization and concentration of complexes in them progress simultaneously, perhaps each enhancing the other. 5. Finally, antigen and antigen-antibody complexes are eliminated from the circulation; then the lesions of serum sickness promptly burn themselves out and are repaired and in the process the antigen and antibody in them are catabolized.[26,27]

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Pathologic Observations Concerning the Kidney in Progressive Systemic Sclerosis

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The recognition that scleroderma may represent only one manifestation of a systemic disease, aptly designated by Goetz [1] as progressive systemic sclerosis (P. S. S.), has prompted investigation of the visceral lesions of this malady. Systemic studies have clearly defined the cardiovascular and gastrointestinal alterations, but until recently little emphasis or significance has been attributed to renal involvement. Indeed, Beerman,[2] in his comprehensive review of P. S. S. in 1948, cited only the two cases recorded by Bevans [3] to illustrate renal changes which may be encountered in this disease. More recently, Moore and Sheehan [4] have described the occurrence of mucoid swelling of the intima of interlobular renal arteries and fibrinoid necrosis of distal portions of these vessels, including afferent arterioles and occasionally glomerular tufts, in three patients with P. S. S. who died from renal failure. They concluded from their observations, as well as from information extracted from previously recorded cases, that these renal lesions represent a specific morphologic entity attributable to P. S. S. A similar view is expressed in the reports of Swarm and Germuth,[5] Bartels et al.,[6] Bagnall and Robinson,[7] and Hannigan, Hannigan, and Scott.[8] Calvert and Owen,[9] although referring to a "true scleroderma kidney," emphasized that the lesions may also be characterized by alteration of glomerular basement membranes similar to that seen in lupus erythematosus. That such an overlap in the morphologic manifestations may prevail in these diseases is apparent from the cases of P. S. S. studied by Bevans,[3] Platt and Davson,[10] Allen,[11] Conestabile and Natale,[12] Enticknap,[13] Pollack,[14] and Banks.[15]

On the other hand, some investigators [11, 16,17] have been impressed by the similarity of the renal lesions described by Moore and Sheehan [4] as characteristic of P. S. S. to those encountered in patients with malignant nephrosclerosis. This observation may be significant concerning the pathogenesis of the renal lesions encountered in patients with P. S. S. exhibiting renal failure and hypertension.

It was considered worth while to investigate and compare the morphologic features of kidneys obtained from patients with P. S. S. with those of malignant nephrosclerosis. The application of various histochemical techniques was utilized in an attempt to appreciate differences, if any, which might not be apparent in sections stained by routine oversight methods.

Material and Methods

Paraffin blocks and sections prepared from the kidneys of 11 patients with P. S. S. and 12 patients dying of accelerated or malignant nephrosclerosis, as well as wet formalin-fixed tissue from 3 examples of the former and 8 of the latter, were available for study. The case records of the patients with P. S. S. are epitomized in Table 1. The examples of malignant nephrosclerosis included six patients in whom there was neither antecedent history of hypertension nor histologic evidence of benign or chronic nephrosclerosis, four in whom such evidence was apparent and who died of acceleration of their disease, and two patients in whom evidence of accelerated nephrosclerosis occurred during the course of proliferative glomerulonephritis. Sections from both groups were stained

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TABLE 1.—*Clinical Findings in Patients with P. S. S.*

Case	Age	Sex	Duration Illness, Yr.	Adrenal Steroid Therapy	Terminal Blood Pressure Mm. Hg	Funduscopic Findings *	Urine Examination		Terminal Blood NPN or BUN, Mg./100 Cc.
							Protein	Casts	
1	66	M	3½ mo.	No	210/110	N, E	2+	Granular	180 (NPN)
2	50	M	2	No	220/120	N, H, E	1-3+	Granular Hyaline Red cell	105 (BUN)
3	45	F	1	Yes	180/110	N, H, E, P	1-3+	Granular Hyaline	167 (BUN)
4	47	F	2	Yes	170/110	H, E, P	2-4+	Granular Waxy Hyaline	211 (NPN)
5	54	M	2	Yes	148-190/110-140	(Not examined)	3+	Granular	92 (BUN)
6	34	F	1½	No	114/76 †	Normal †	Tr.	----------	25 (NPN) †
7	36	M	4	Yes	130-160/90-110	Normal ‡	1+	Hyaline	35 (NPN) ‡
8	33	M	3½	Yes	120/80 §	Normal §	75 mg./100 Cc.	Hyaline	72 (NPN)
9	66	F	16	Yes	120-158/78-92	N	50 mg./100 Cc.	----------	49 (NPN)
10	42	F	16	Yes	149-80/80-60	N	0-30 mg./100 Cc.	----------	32-46 (NPN)
11	49	M	7	Yes	90/?	(Not examined)	Tr.	----------	30 (NPN)

* N indicates narrowing of vessels; H, hemorrhage; E, exudate; P, papilledema.
† 1 mo. before death.
‡ 2 mo. before death.
§ 6 mo. before death.

simultaneously according to the following methods as described in Lillie's "Histopathologic Technic and Practical Histochemistry,"[38] unless otherwise indicated:

1. Hematoxylin and eosin

2. Oil red O for demonstration of lipid on frozen sections cut at 10μ

3. Periodic acid Schiff, hematoxylin with and without antecedent digestion with barley-malt diastase (Fisher lot No. 530052) 0.1% in pH 6 phosphate buffer, 1 hour at 37 C.

4. Ritter-Oleson technique

5. Rinehart-Abul-Haj method for mucopolysaccharides

6. Alcian blue according to the method of Mowry[39] for acidic carbohydrates

7. Thionin, 1:10,000 pH 4 for ½ hour

8. Treatment of sections with testicular hyaluronidase (Wyeth lot No. 162603) (100-200 TRU per milligram), 100-200 units per cubic centimeter, pH 5.5 acetate-acetic acid buffer 1 hour at 37 C followed by 3, 5, 6, 7

9. Treatment of sections with streptococcal hyaluronidase (Wyeth)* (20 TRU per milligram), 200 units per cubic centimeter, pH 5.5 acetate-acetic acid buffer for 1 hour at 37 C followed by 3, 5, 6, 7

10. Wilder reticulum method

11. Masson trichrome

12. Mallory's phosphotungstic acid hematoxylin after mordanting sections in saturated sublimate for 3 hours at 72 C

13. Treatment of sections with trypsin (Difco lot No. 428446) in phosphate buffer, pH 7.6 for 2, 4, and 24 hours at 37 C followed by 3, 9, 10, and 11

14. Verhoeff-Van Gieson for elastic fibers, collagen, and smooth muscle.

Results

Progressive Systemic Sclerosis.—In two patients (Cases 6 and 10) no renal lesions were apparent.

* Drs. Funk and Warren, of Wyeth Laboratories, Inc., supplied the streptococcal hyaluronidase.

TABLE 2.—*Correlation of Pathologic Findings and Blood Pressure and Renal Failure in Patients with P. S. S.*

Case	Organs Involved Post Mortem *	Intimal Change (Interlobular Artery) †	Vascular Fibrinoid †	Involved Glomeruli, % ‡	Terminal Blood Pressure	Terminal NPN or BUN, Mg./100 Cc.
1	S, E, M, K	3+	3+	54%	210/110	180 (NPN)
2	S, H, K	3+	3+	62%	220/120	105 (BUN)
3	S, H, K	3+	3+	60%	180/110	167 (BUN)
4	S, H, K	2+	2+	50%	170/110	211 (NPN)
5	S, H, E, L	2+	2+	55%	148-190/110-140	92 (BUN)
6	S, H, M, K	2+	2+	53%	114/76 §	25 (NPN) §
7	S, K	2+	1+	42%	130-160/90-110	35 (NPN) ≠
8	S, H, E, L, K	1+	±	25%	120/80 ¶	72 (NPN)
9	S, E, K	±	±	3%	120-158/78-92	49 (NPN)
10	S, H, E	----	----	----	140-80/86-60	32-46 (NPN)
11	S, H, E, L	----	----	----	90/?	30 (NPN)

* S indicates skin; H, heart; E, esophagus; L, lung; M, muscle; K, kidney.
† Extent of changes: 3+, marked; ±, rare.
‡ Includes ischemic thickening of basement membrane.
§ 1 mo. prior to death.
≠ 2 mo. prior to death.
¶ 6 mo. prior to death.

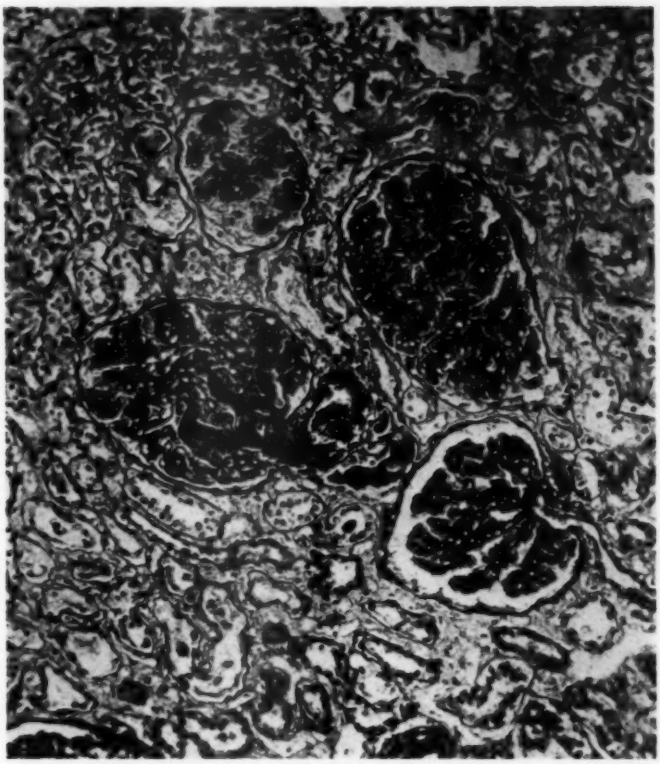

Fig. 1.—Section from Case 2, revealing thickened glomerular basement membranes as well as ischemic change. Fibrinoid degeneration of an afferent arteriole is evident, with extension of material into glomerular tufts and also extracapillary space. Distortion and degenerative changes in tubules are also apparent. Periodic acid Schiff; × 145.

The kidneys from the remaining nine patients with P. S. S. in all except one (Case 7) appeared larger than normal. Their combined weights varied from 310 to 360 gm. The cortical surfaces were smooth except for the presence of small infarcts observed in eight instances. Petechiae were not infrequent. In two cases the surfaces were finely granular, and the combined weight of the kidneys in one of these was only 180 gm. The cut surfaces revealed a pale cortex containing varying numbers of miliary infarcts and petechiae demarcated from congested medullary tissue.

Although the lesions in each case were for the most part similar microscopically, the degree of renal involvement varied. The percentage of involved glomeruli ranged from 3% to 62% (Table 2). Glomerular lesions were focal and consisted principally of localized or diffuse thickening of basement membranes, particularly that portion stained with the periodic acid-Schiff sequence of the Ritter-Oleson technique. The membranes were frequently undulated or of the ischemic type (Fig. 1). In two instances wire-loop changes were observed in the basement membranes indistinguishable from those seen in lupus erythematosus (Fig. 2). The glomeruli of severely involved kidneys revealed homogeneous and granular eosinophilic masses within capillary lumens. In some instances there was direct extension of this material from afferent arterioles (Fig. 1). Many glomerular capillaries appeared replaced by this material, which, for the most part, displayed the tinctorial properties and enzymatic resistance which one of us (E. R. F.) [20] has previously recorded as characteristic of fibrinoid: notably, periodic acid-Schiff-positive; orange and orange-blue with phosphotungstic acid, hematoxylin; argyrophilic; purple with the trichrome procedure, and resistant to tryptic digestion. In

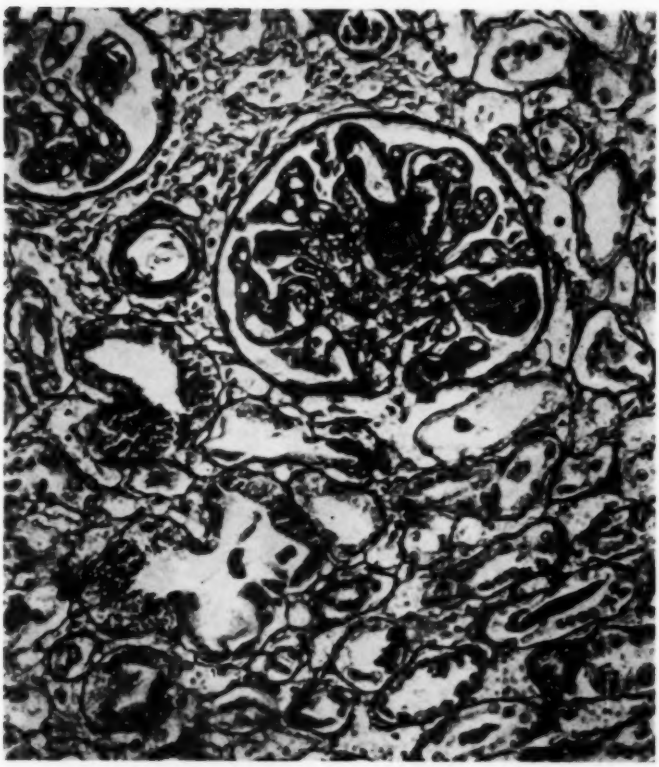

Fig. 2.—Section from Case 3, revealing wire-loop change in glomerulus. Hyaline-droplet formation in epithelium of proximal convoluted tubules also present. Periodic acid Schiff; ×265.

Case 1 focal proliferation of glomerular endothelial cells was observed (Fig. 3). Hyaline-droplet formation in glomerular epithelial cells was conspicuous in those kidneys revealing similar alteration in the tubular epithelium. So-called hematoxylin bodies were not observed. Bowman's capsules were not conspicuously altered, and obsolete glomeruli were rare. The cortical infarcts observed grossly appeared as poorly staining cortical substance. Such infarcted areas were frequently hemorrhagic, and inflammatory infiltrate was observed in such zones only occasionally.

A variety of alterations was noted in the tubules. In severely affected kidneys there were marked degenerative changes characterized by flattening and hyperchromasia of epithelial cells of convoluted tubules. The lumens of such tubules were frequently dilated and contained hyaline and granular casts. Hyaline-droplet formation was also evident in the epithelial cells of proximal convoluted tubules (Fig. 2). The basement membranes of involved tubules were frequently thickened and distorted. The intertubular stroma was frequently thickened by fibrous tissue containing varying degrees of lymphocytic and plasma-cell infiltrate. The collecting tubules were relatively uninvolved except for the presence of casts within their lumens. The ground substance of the medulla appeared unaltered.

Interlobar vessels were normal except for the rare occurrence of an arteriosclerotic plaque of the fibrous intimal type (Fig. 4). Interlobular arteries, on the other hand, revealed moderate to marked luminal narrowing due to relatively acellular intimal deposits of metachromatic Rinehart-and-alcian-blue-positive material (Fig. 5). Treatment with both streptococcal and testicular hyaluronidase resulted in the inhibition of staining with the former two methods but

Fig. 3.—Glomerulus from Case 1, illustrating moderate endothelial proliferation. Hematoxylin and eosin; × 235.

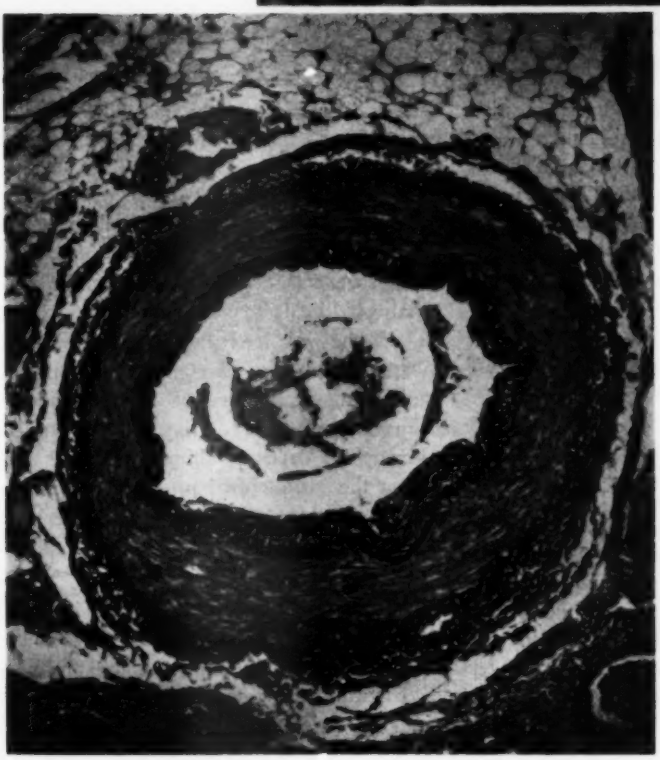

Fig. 4.—Interlobar artery of kidney from Case 2. There is only slight reduplication of internal elastic lamina, with focal intimal sclerosis of mild degree. Verhoeff-Van Gieson; × 60.

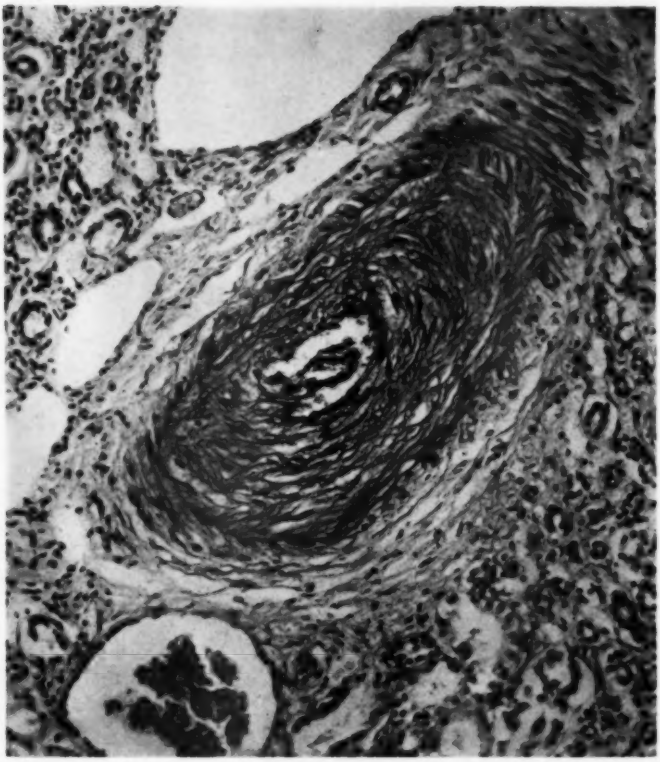

Fig. 5.—Interlobular artery from Case 3, revealing pronounced intimal accumulation of acid mucopolysaccharide (appearing gray). Thionin; × 135.

only partial inhibition of Rinehart-positive material. In addition, fine reticulum fibers were noted within these intimal cushions, and in some instances material with the tinctorial properties of collagen was also identified (Fig. 6). Fibroglial fibers could be identified by the phosphotungstic acid and hematoxylin (PTAH) technique in those instances in which this intimal change contained spindle-shaped cells. For the most part the internal elastic membrane of these altered vessels revealed only slight reduplication. More distal ramifications of the interlobular arteries (prearterioles) frequently revealed intimal lamination. Fibers giving rise to this appearance stained as collagen rather than elastica, although the latter occasionally could be identified (Fig. 7). The interstices of the laminations frequently revealed mucopolysaccharide identical to that comprising the intimal alteration of larger vessels. Afferent arterioles in severly damaged kidneys frequently had fibrinoid degeneration of their media with intraluminal thrombi of similar material. This change, as well as similar alterations within glomeruli, was unaccompanied by inflammatory infiltrate. In those instances in which suitable tissue was available, sudanophilic material could be demonstrated in many but not all of the involved interlobular arteries. When present this was evident in the media, and to a smaller extent in the intima. Damaged arterioles frequently revealed sudanophilic deposits within their walls and within the intraluminal masses as well. In an occasional case rare hyalinized arterioles resembling those observed in benign nephrosclerosis were noted.

Close correlation was obtained between the degree of involvement of the interlobular arteries of these kidneys and the presence of cortical infarction. Glomerular and ar-

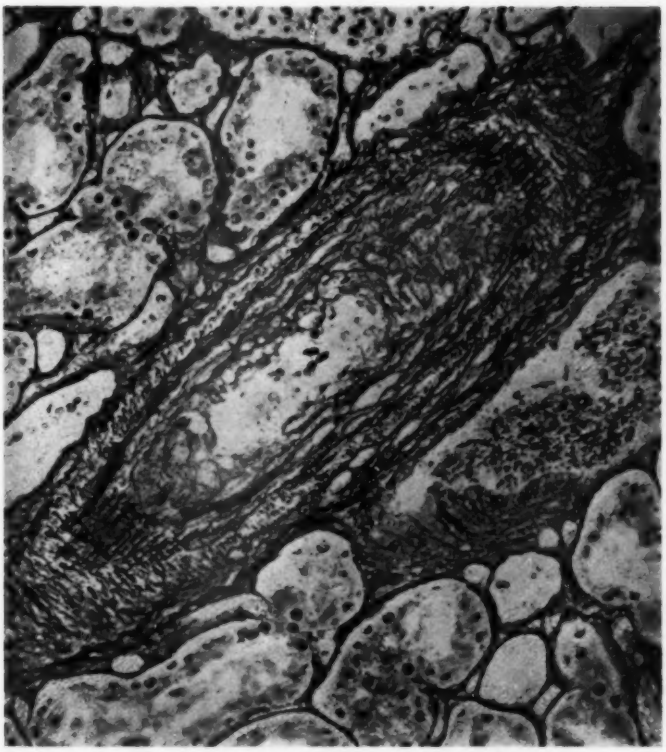

Fig. 6.—Interlobular artery from Case 3, revealing reticulum fibers in intima. Wilder reticulum stain; × 175.

teriolar damage was less frequent than alterations of the interlobular vessels.

Malignant Nephrosclerosis.—The gross and microscopic features of the kidneys from patients with malignant or accelerated nephrosclerosis were similar to the findings recorded in detail by others.[11,21,22] Glomerular, tubular, and vascular lesions were encountered in these kidneys, which were morphologically and tinctorially identical to those noted in the kidneys from patients with P. S. S. Differentiation was possible only in those instances in which proliferative glomerulonephritis or preexisting hypertension with arteriolar nephrosclerosis represented antecedent disease states. In this latter group frequent obsolete glomeruli, thickening of Bowman's capsule, and hyalinization of afferent arterioles were features attesting to the chronicity of the underlying renal disease.

Clinicopathologic Correlation.—The onset of renal failure and hypertension in five of the cases noted in Table 1 occurred from several days to four weeks prior to death. A high correlation between the severity of renal involvement as indicated by the degree and extent of vascular and glomerular damage and the manifestations of renal failure including hypertension was present in all but two instances (Table 2). It appears significant that in these latter no clinical data concerning blood pressure or renal function were available, except that obtained one and six months prior to death. It is also noteworthy that no renal lesions were encountered in the two patients who were adequately observed to be free from hypertension or renal failure.

Relationship of Renal Lesions in Patients with Progressive Systemic Sclerosis to Therapy.—No direct correlation was noted

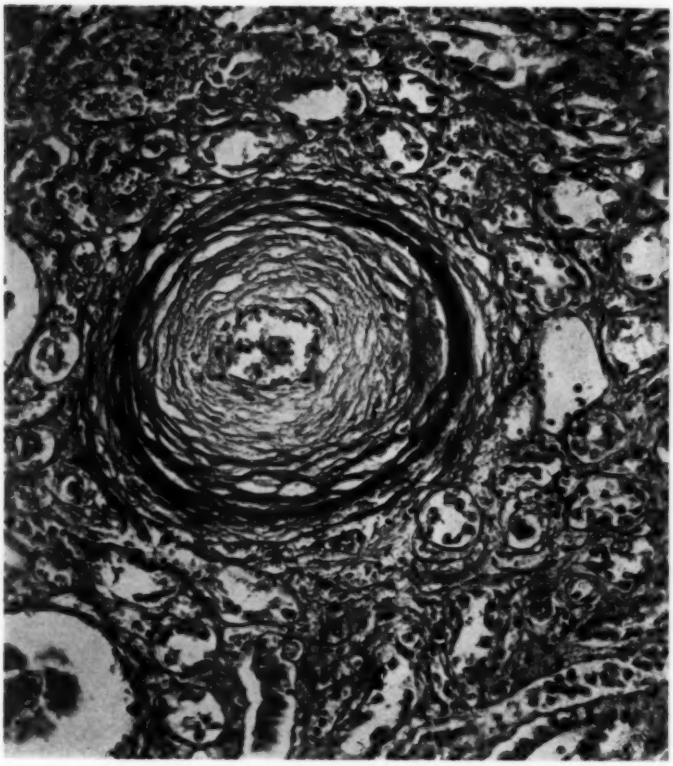

Fig. 7.—Distal ramification of interlobular artery (prearteriole), revealing moderate reduplication of elastica. The intimal change is comprised of collagen fibers separated by ground substance (not apparent). Verhoeff elastic stain; × 185.

between the type of therapy utilized in the management of these patients and the extent of renal involvement. Two patients in whom adrenal steroid therapy was utilized failed to reveal such change, and two in whom these agents were not employed had severe lesions.

Comment

The morphologic findings in this study confirm previous descriptions of the renal lesions observed in patients with P. S. S. dying from renal failure. It appears that the extent of vascular and glomerular involvement may be correlated in those instances in which sufficient clinical data are available with the severity of renal failure and/or hypertension. Minimal renal involvement may not be accompanied by clinical manifestations of renal insufficiency, for the renal lesions are predominantly focal.

However, we have found no significant morphologic or tinctorial differences between the renal lesions observed in patients with P. S. S. terminating in renal failure and those encountered in a group of cases of malignant nephrosclerosis. The "wire-loop" alteration of the glomerular basement membranes seen in some examples of P. S. S. was not unlike that observed more commonly in systemic lupus erythematosus. In addition, proliferation of glomerular endothelial and epithelial cells, which is another feature of the renal involvement of lupus erythematosus, was noted in one case (Case 1). Although these findings suggest an overlap in the morphologic aspects of P. S. S., it should be noted that hematoxylin bodies, common in lupus erythematosus, were not encountered in these kidneys from patients with P. S. S. and that the type of vascular lesions encountered in the latter do not con-

stitute a conspicuous feature of the renal alterations in systemic lupus erythematosus. These considerations indicate the difficulty in relating any degree of morphologic specificity to the renal lesions of P. S. S.

The appearance of the vascular and glomerular lesions in the kidneys of those patients with P. S. S. having renal failure reflects the acuity of the renal involvement in this disorder. Focal cortical infarction, undulation of glomerular basement membranes, and lack of glomerular obsolescence have been emphasized by McManus [23] to be indicative of acute renal ischemia. The presence of ground substance, and in many instances reticulum fibers and fibroblasts in the intimal lesions of the interlobular arteries, may represent an early stage in the development of renal arterial and arteriosclerosis. Such components have been considered essential for the formation of collagen.[24,25] The acid mucopolysaccharide constituting these "edematous" arterial lesions in both P. S. S. and malignant nephrosclerosis may be identified by its lability to streptococcal and testicular hyaluronidase as being nonsulfated or of the hyaluronic acid type. The lack of complete removal of Rinehart-positive material by these enzymes may be attributed to the likelihood that this technique colors other substances, notably collagen, as well as mucopolysaccharide. It is of interest that the acid mucopolysaccharide encountered in atherosclerosis and apparently in normal vessels has been considered to be of the sulfated variety.[25] It cannot be concluded at this time whether this variation reflects a functional difference in the ground substance in these apparently dissimilar disorders.

The pathogenesis of these renal lesions in patients with P. S. S. has not been conclusively elucidated. It has been suggested [7,8,26] that the vascular lesions may represent a manifestation of the scleroderma-tous process per se and that such changes are responsible for the development of hypertension and renal failure. It is to be noted, however, that the intimal mucoid change observed in the interlobular arteries is not seen in other viscera or the skin in P. S. S. and that fibrinoid necrosis is only rarely observed in such sites in the absence of renal involvement. It seems unlikely that the arteriolar and glomerular lesions represent the primary site of renal involvement. Although their fibrinoid nature suggests that they may represent a manifestation of hypersensitivity, there is little evidence at present to implicate such a mechanism in P. S. S. or in malignant nephrosclerosis. Further, the occurrence of these lesions appears to be dependent upon the vascular alteration observed in larger interlobular arteries. As noted by others,[27] there does not appear to be any direct relationship between the administration of adrenal steroids and the appearance of these renal manifestations. Whether or not such therapeutic agents may accelerate or aggravate existing alterations requires further clinical and experimental information.

It has also been proposed that the vascular alterations within the kidney may be the result of spasm of renal vessels analogous to the frequent occurrence of Raynaud's phenomenon in peripheral vessels of patients with P. S. S.[17] Indeed, similarity between the morphologic appearance of the latter and the renal arteries, presumably those characterized by laminated intimal sclerosis, has been noted. That vasospasm may result in vascular necrosis has been demonstrated experimentally by the administration of epinephrine,[28] and Williams [29] has recently described intimal proliferation in the distal portions of partially occluded vessels. In the light of the morphologic and tinctorial similarity between the renal lesions in P. S. S. and malignant nephrosclerosis it appears significant to recall that a similar mechanism—renal vasospasm—has been considered in the pathogenesis of the latter.[21] The tendency for patients with P. S. S. to develop such vascular phenomena may account for the apparently greater than fortuitous association of malignant nephrosclerosis with this disease.

Although it is generally agreed that vascular lesions within the kidney may aggravate a preexisting hypertension, it has not been established unequivocally that such lesions are essential for the initiation of hypertension in malignant or benign nephrosclerosis. Evidence that hypertension per se may induce severe vascular lesions of the type observed in P. S. S. is well recognized.[30] Unfortunately, the role of hypertension in the pathogenesis of the renal lesions in P. S. S. has been minimized because of the absence of hyperpiesis in some paients in whom severe renal lesions were encountered at necropsy. Scrutiny of such cases, however, discloses a paucity or lack of sphygmomanometric determinations, particularly during terminal episodes of the patient's illness. The importance of complete clinical information is emphasized by the acuity of the renal lesions and the observation of hypertension occurring only days prior to death in several patients in this study. Furthermore, the results of careful clinicopathologic studies in essential hypertension [22] would indicate that in all likelihood hypertension was present in those instances in which renal failure was accompanied by severe vascular lesions. The high correlation between the extent of renal involvement and severity of hypertension noted in this study further suggests such an event.

It becomes evident from the foregoing considerations that the problem of the pathogenesis of the renal lesions occurring in some patients with P. S. S. appears similar to that of malignant nephrosclerosis, from which disease it is morphologically and tinctorially indistinguishable. Although the concept of renal vasospasm appears plausible in accounting for the renal lesions and clinical manifestations observed, it appears equally tenable at present that such renal involvement may be the result of hypertension per se. This latter may be the result of generalized vasospasm, a concept also proposed for the pathogenesis of essential hypertension. Both views are in keeping with the well-recognized occurrence of vasospastic phenomena in patients with P. S. S. Utilization of renal biopsy would appear invaluable in establishing the primacy of either the renal lesions or hypertension in the development of this form of renal disease.

Summary

The renal lesions encountered in 9 of 11 patients with progressive systemic sclerosis (P. S. S.) are described and noted to be morphologically and tinctorially indistinguishable from those encountered in the kidneys from 12 patients with malignant nephrosclerosis. The intimal "mucoid" of the interlobular arteries in both diseases is characterized as acid mucopolysaccharide of the hyaluronic acid type. Evidence indicates that this vascular change may represent an early stage or acute phase in the development of the more familiar vascular hyalinization noted in chronic forms of nephrosclerosis.

The extent and severity of the renal lesions in patients with P. S. S. may be correlated with the degree of renal failure and/or hypertension exhibited by such patients when sufficient clinical data allowing for such a relationship is available. These clinical manifestations may occur precipitously and shortly prior to death, requiring at times close clinical observations for their recognition.

The problem of the pathogenesis of these renal alterations in patients with P. S. S. is discussed. It is concluded that either renal vasospasm or generalized vascular hypertonicity resulting in hypertension may account for the renal lesions and clinical manifestations encountered. Each appears compatible with the well-recognized occurrence of vasospasm in patients with P. S. S. and may explain the more than fortuitous association of malignant nephrosclerosis with this disease.

Drs. Bernard Fetter, George Schreiner, Thomas Moran, and Leonard Meyers provided some of the pathologic material utilized in this study.

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Atherosclerosis in Rabbits After Intravenous Injection of Colloidal Solutions

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In the course of experiments on the reticuloendothelial system various investigators have observed the effect of splenectomy or of the intravenous injection of agents thought able to block the reticuloendothelial system on the serum lipids. Hyperlipemia, hypolipemia, and normal lipemic states have been reported in these experiments, and the variable results have led to conflicting conclusions with regard to the effects both of the blocking agents [1-7] and of splenectomy.[3,5,8-12] The present paper reports the occurrence of a transient hyperlipemia and hypercholesteremia in rabbits following the intravenous injection of colloidal thorium dioxide (Thorotrast) or methylcellulose (Methocel). In addition, the occurrence of small atherosclerotic lesions of the aortic intima in some of the treated rabbits is reported.

Materials and Methods

General.—Fifty-seven New Zealand white rabbits of both sexes between 3 months and 1 year old were used. Approximately 12 to 15 ml. of blood was obtained from the ear for analysis, and chemical estimations were made of the concentration in the serum of free and total cholesterol,[13] lipid phosphorus,[14] and total fatty acids [15]; the fatty acids of neutral fat were calculated.[16]

Certain rabbits were given injections of colloidal thorium dioxide. It was warmed to about 35 C and was injected during a period of one-half to one minute into the marginal ear vein. The colloidal thorium dioxide used had been purchased from the Heyden Chemical Corporation, New York, in 1951 and was used in 1952. Subsequently, colloidal thorium dioxide was obtained from the Testagar Company, Detroit, and used in somewhat similar experiments in 1956. The latter product elicited only a mild hyperlipemic response in only one of six rabbits observed, whereas the former product had a definite hyperlipemic effect in about 85% of rabbits given injections, in both 1952 and 1956. No further tests of the second product have been made.

On the completion of an experiment the rabbits were killed, and complete autopsies were done. The endothelium and intima of the aorta, together with a little subjacent media, were prepared by the surface technique of Lautsch, McMillan, and Duff.[17] In this way the intact aortic intima from the ascending arch to below the renal arteries was mounted on slides with the endothelial surface uppermost and examined microscopically.

EXPERIMENT I.—Thirty-six rabbits were studied. They comprised nine groups; each group contained four littermate animals. Both male and female rabbits were included in each litter. All rabbits had been weaned at least six weeks prior to the experiment.

From each litter of four animals one rabbit was fed cholesterol; it was given access to 1 gm. of cholesterol daily with 6 gm. of corn oil and 93 gm. of rabbit pellets. The second rabbit received a similar daily ration but was also given an intravenous injection of colloidal thorium dioxide, receiving a single injection of 3 ml. per kilogram of body weight. The third rabbit was fed a ration of ordinary pellets without added cholesterol or oil and given an injection of colloidal thorium dioxide in the same way as the second animal. The fourth rabbit was fed ordinary rabbit food and was not given an injection.

Chemical estimations of the lipid content of the serum were made prior to the feeding of cholesterol and to the injection of colloidal thorium dioxide. They were also made daily during the course of an experiment and at its termination, as was appropriate.

Four of the nine litters were fasted for 16 hours to increase the rabbits' appetite and then fed

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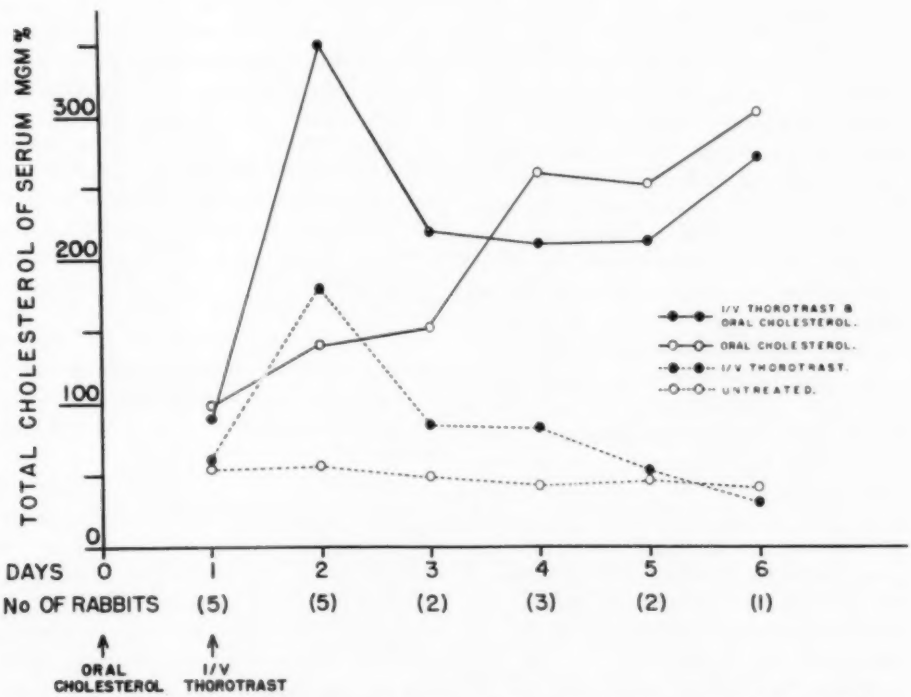
† Dr. Duff died Nov. 1, 1956.

ordinary or cholesterol-containing pellets; two hours after the exhibition of food the appropriate rabbits were given injections of colloidal thorium dioxide in the manner described above. The litters were killed 4, 8, 16, and 24 hours after the beginning of the experiment. The five remaining litters were similarly fasted and fed. Twenty-four hours after the exhibition of food the appropriate rabbits were given injections of colloidal thorium dioxide. These litters were killed two, three, four, five, and six days after the start of the experiment.

EXPERIMENT II.—For a second experiment 15 rabbits were studied. They were fed ordinary pellets without added cholesterol or corn oil. Four rabbits were given a single intravenous injection of 25 ml. of a 1.2% W/V aqueous solution of methylcellulose (Methocel 1500 cps., Dow Chemical Co.). Six rabbits were given intravenous injections of a total of 50 cc. of a 0.5% W/V solution of methylcellulose dissolved in isotonic saline, using divided doses of 20 ml., 20 ml., and 10 ml. at intervals of one hour. Five rabbits were given injections once intravenously with 50 ml. of isotonic saline. Two of the rabbits given injections of 1.2% methylcellulose died. The 13 surviving rabbits were killed on the eighth experiment day. Chemical estimations of serum lipids and microscopic examination of the aorta were done as described for Experiment I.

EXPERIMENT III.—For a third experiment six rabbits were fed an ordinary diet of pellets without added cholesterol or corn oil and were given intravenous injections of colloidal thorium dioxide. It was injected in a quantity of 2 ml. per kilogram of body weight once every four or five days for three weeks. The dose was then increased to 2.5 ml. per kilogram of body weight, and the injections were continued for an additional six and one-half weeks. In all, each rabbit received 15 injections during a period of 66 days. Three rabbits were killed on the 78th day of the experiment, and the remaining three were killed on the 90th day. Chemical estimations of the lipids of the serum were made immediately before and 24 hours after each injection. The aorta was examined as described above.



Graph 1.—This graph illustrates the average effect on the serum total cholesterol of littermate rabbits of feeding cholesterol, of injecting colloidal thorium dioxide intravenously, and of combining both procedures. The illustrated experiment included 20 rabbits (i. e., five litters of four rabbits each including the control animals); one litter was killed each day after the second day for five days. The transient hypercholesterolemic effect of the intravenous injection of colloidal thorium dioxide is clearly shown.

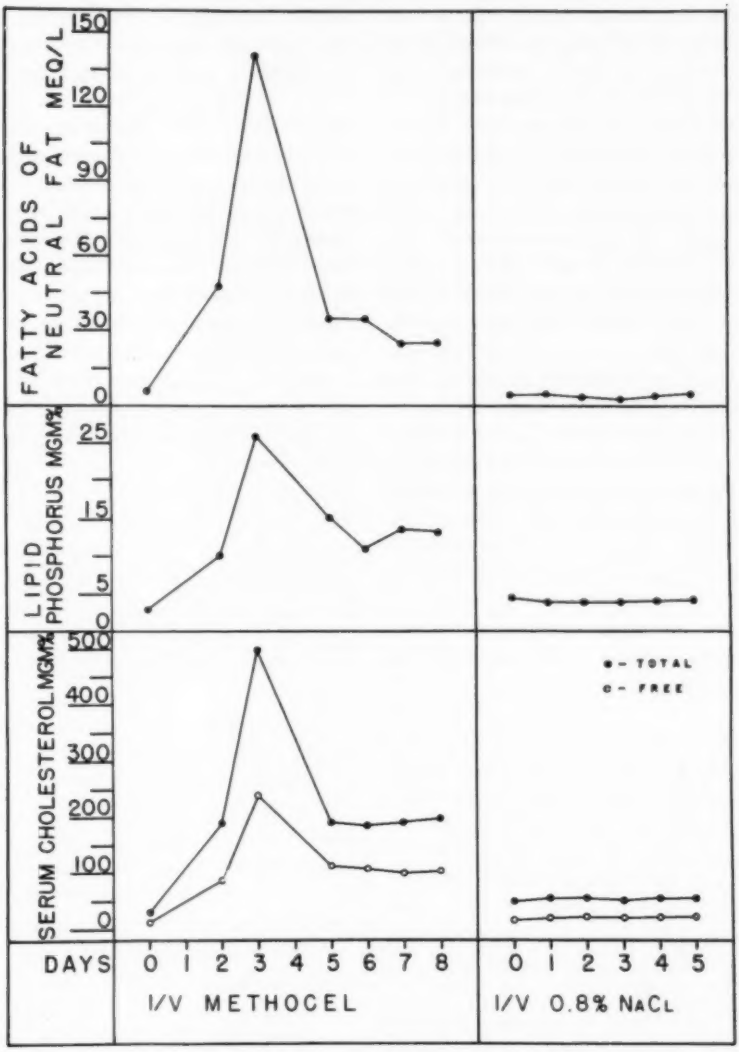
Results

EXPERIMENT I.—The results of the chemical analyses of the serum total cholesterol of the five litters killed on days two, three, four, five, and six, respectively, are summarized in Graph 1. Similar results were obtained from the analyses for serum lipid phosphorus and total fatty acids. It was observed that there was no abnormal deviation in the serum lipids of the normal control rabbits fed ordinary food. There was an abrupt but moderate and transient increase in the concentration of serum cholesterol in the rabbits given injections of colloidal thorium dioxide but fed ordinary food without added cholesterol or oil. The increase was greatest in the sample taken 24 hours after injection. There was a progressive increase in the serum cholesterol of the rabbits fed cholesterol and oil but not given injections of colloidal thorium dioxide. In the rabbits receiving both cholesterol and colloidal thorium dioxide the serum lipids increased quickly to the highest concentrations observed, decreased subsequently, and then increased again. The form of the curve of concentration of serum total cholesterol of the rabbits fed cholesterol and given injections of colloidal thorium dioxide represents approximately the combination of the effects observed on the serum lipids of feeding cholesterol and oil alone or of injecting colloidal thorium dioxide alone (Graph 1).

No appreciable effect of the experimental procedures on the serum lipids was noted in the litters killed four or eight hours after the start of the experiment. In the litter killed 16 hours after the start of the experiment (and 14 hours after the injection of colloidal thorium dioxide) the serum total cholesterol concentration was increased in the rabbit given injections of colloidal thorium dioxide from a normal of 48 mg. per 100 cc. to 160 mg. per 100 cc. The lipid phosphorus and total fatty acids of the serum were also increased. However, the rabbit in this litter that received both cholesterol and colloidal thorium dioxide did not manifest any changes in the serum lipids. In the litter killed 24 hours after the start of the experiment (and 22 hours after the injection of colloidal thorium dioxide) the terminal serum total cholesterol concentration in the rabbit fed cholesterol and oil was 82 mg. per 100 cc., in the rabbit given injections of colloidal thorium dioxide it was 110 mg. per 100 cc., and in the rabbit receiving both cholesterol and colloidal thorium dioxide it was 170 mg. per 100 cc. The normal concentrations in these animals had been about 45 mg. per 100 cc.

Microscopic examination by the surface technique of the aortas disclosed no atherosclerotic lesions in any of the aortas of the control rabbits that received ordinary food only. Five of the aortas of the nine rabbits that were given injections of colloidal thorium dioxide showed atherosclerotic lesions; the aorta of the rabbit from the three-day litter could not be examined. The aortas of eight of the nine rabbits fed cholesterol and oil had atherosclerotic lesions, while seven of the nine aortas of the rabbits that received cholesterol and oil together with colloidal thorium dioxide showed atherosclerotic lesions. The lesions contained lipid and were similar to those described by Duff, McMillan, and Ritchie [18] in rabbits fed cholesterol. In addition to the lipid, the lesions in the rabbits that received colloidal thorium dioxide also contained particles of this material within macrophages in the manner described by Duff, McMillan, and Lautsch.[19] The apparent occurrence of extracellular fat as described by Ritchie, Macklem, and Weigensberg [20] was not observed.

EXPERIMENT II.—Of the 10 rabbits fed ordinary food without added cholesterol or oil and given intravenous injections of methylcellulose, two died within a few hours. They had received a single injection of 25 ml. of a 1.2% weight per volume aqueous solution. Five of the eight surviving rabbits did not manifest hyperlipemia, two developed prominent but transient hyperlipemia, and one showed only a mild transient increase in the concentrations of the serum lipids. The striking hyperlipemia observed



Graph 2.—This graph illustrates in one rabbit the transient hyperlipemic effect of the intravenous injection of a solution of methylcellulose.

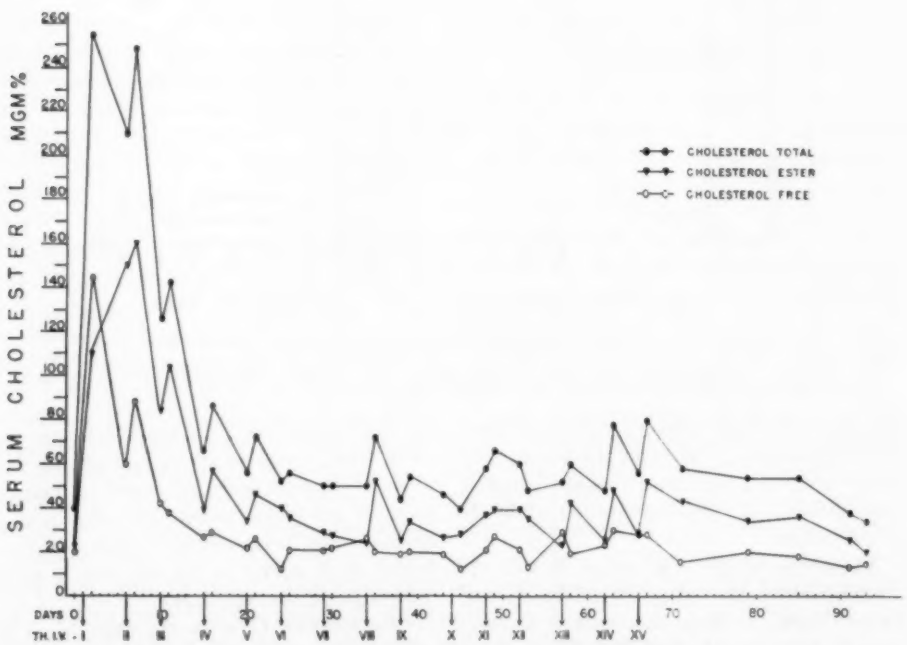
in one of the three affected rabbits is illustrated in Graph 2. None of the five rabbits given injections of isotonic saline showed a disturbance in the concentration of lipids in the serum.

The aortas of the three rabbits that developed hyperlipemia had small atherosclerotic lesions similar to those described in Experiment I. The macrophages in the lesions contained lipid and also material thought to be methylcellulose. No atherosclerotic lesions were found in the aortas of the five rabbits that were given injections of methylcellulose but which failed to develop hyperlipemia, nor were atherosclerotic lesions found in the aortas of the five rabbits given injections of isotonic saline.

EXPERIMENT III.—In the experiment in which six rabbits were fed an ordinary diet without added cholesterol or oil and

were given intravenous injections of colloidal thorium dioxide at semiweekly intervals for many weeks, a moderate and transient increase in the concentrations of the serum lipids was observed in five of the six animals following the initial injection of colloidal thorium dioxide. The sixth animal developed only a relatively slight hyperlipemia. During the interval of four or five days until the next injection was given the serum lipid concentrations became almost normal. Second and third episodes of transient hyperlipemia followed the second and third injections of colloidal thorium dioxide, but the increase in the concentrations of lipids was less on each subsequent occasion. The concentrations of lipids in the serum remained at normal or slightly increased levels throughout the remainder of the experiment. The serum cholesterol concentrations of one rabbit are illustrated in Graph 3.

The aortas of all six rabbits showed focal atherosclerotic lesions. The areas of the intima involved by lipid deposits were extensive in all animals. Relatively large areas surrounding and including the obliterated orifice of the ductus arteriosus, the lips of the orifices of the intercostal arteries, and the large arteries of the neck and abdomen were the sites of large lesions. The lesions were not thick, being only three or four layers of cells in depth. Macrophages with both colloidal thorium dioxide granules and lipid droplets in their cytoplasm were scattered in considerable numbers in the subendothelial layers of the aortic intima, and among the macrophages there were numerous fibroblasts which contained both lipid and colloidal thorium dioxide. Many of the cells containing colloidal thorium dioxide appeared to have suffered some nuclear degeneration. The nuclei of these cells frequently showed marked distortion and some fragmentation. Lobules of nuclear material were often joined to the main nuclear mass by only an attenuated thread of chromatin. Other nuclei had the form of an elongated



Graph 3.—This graph illustrates in one rabbit the transient, tachyphylactic, hypercholesterolemic effect of repeated and frequent intravenous injections of colloidal thorium dioxide.

irregular ribbon with a wrinkled nuclear membrane. Similar nuclear changes have been described in the spleens of rats given intravenous injections of large amounts of colloidal thorium dioxide.[21] As previously reported,[19] a considerable number of endothelial cells overlying the atherosclerotic lesions also showed accumulations of colloidal thorium dioxide granules. The latter were most frequently accumulated in prominent clumps at one or both poles of the nucleus of the endothelial cells.

Comment

The present experiment demonstrates that hyperlipemia, including hypercholesterolemia, can be produced promptly in the rabbit by a single intravenous injection of large amounts of the macromolecular substances, colloidal thorium dioxide and methylcellulose. Colloidal thorium dioxide elicits hyperlipemia in normal rabbits and in rabbits fed cholesterol. If repeated injections are given colloidal thorium dioxide loses much of this effect after three or four injections given at intervals of a few days. A slight increase in the amount of colloidal thorium dioxide injected does not alter this tachyphylactic response after it has been established. The effect of methylcellulose injection on cholesterol-fed rabbits and in prolonged experiments was not tested.

The explanation of the hyperlipemic phenomenon is not apparent from our experimental data, and the literature at hand is not helpful. It would require further and complex investigation to decide whether the effect is primarily attributable to the reticuloendothelial system, to the liver, to the adrenal glands, to physiochemical transport mechanisms in the blood, or, possibly, to the kidney. Any hypothesis must not only explain the immediate transient hyperlipemic effect but must also account for the refractory state of the rabbit that develops in the course of prolonged experimentation. In any case, the hyperlipemic phenomenon demonstrated here may be relevant to the interpretation of experiments in which macromolecular substances are injected and lipid metabolism is observed, as, for example, in the experiment reported by Lequire, Gray, and Cobb,[22] in which the influence of colloidal thorium dioxide on the lipemia-clearing effect of heparin was studied.

Of considerable interest to us was the observation that the intravenous injection of macromolecular substances was associated with lipid deposition and lipophage accumulation in the aortic intima to form atherosclerotic lesions. These microscopic lesions also contained colloidal thorium dioxide or (probably) methylcellulose. Except for the presence of the latter foreign substances the lesions closely resembled the atherosclerotic ones that we are accustomed to demonstrate by the surface technique in rabbits fed cholesterol for a few days.[18] Again, the production of such lesions in this experiment may be germane to the precise interpretation of experiments in which the injection of macromolecular substances is related to the development of arteriosclerotic lesions, as, for example, in the experiments of Hueper,[23,24] in which arteriosclerotic lesions of a special sort were produced in dogs by the intravenous injection of solutions of macromolecular substances.

The atherosclerotic lesions developed by the rabbits given injections also closely resembled those described by Ritchie, Macklem, and Weigensberg[20] in rabbits with hemorrhagic lipemia, although no extracellular lipid accumulations of the sort described by them were seen. The rabbits of the present experiment were bled frequently. While the findings in the control rabbits do not suggest that any disturbance of the serum lipids was caused by the bleeding procedures, it remains possible that some of the increases observed in the concentrations of serum lipids during the experiment may be due, in some small part, to the bleeding.

Summary

Experiments are reported in which the intravenous injection of colloidal solutions of thorium dioxide or of methylcellulose into rabbits resulted in a transient hyper-

lipemia. The affected rabbits developed small atherosclerotic lesions of the aorta.

The experiments reported here were presented in part before the American Society for the Study of Arteriosclerosis.[25]

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The Effect of Alpha-Estradiol Benzoate on Spontaneous and Experimental Atherosclerosis in the Rat

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Introduction

Estrogens, besides having a well-known effect upon genital structures, are also able to influence other tissues; these extragenital actions, however, are not the same in different species. Reports of the literature show that estrogens may affect at least three structures in connection with the problem of atherosclerosis.

(*a*) *The Blood.*—In the chicken, natural or administered estrogens increase blood cholesterol levels as well as the calcium and the phospholipid content.[1] In man, the administration of these hormones decreases the β-lipoproteins and increases the α-lipoproteins, and, by increasing phospholipid levels, they decrease the cholesterol-phospholipid ratio.[1]

(*b*) *The Aorta.*—In the chicken, the administration of diethylbesterol gives rise to atheromatous formation [2] *; on the contrary, estrogens do not influence spontaneous aortic cholesterol deposition in the rabbit,[3] although some observers reported that they may decrease aortic atherosclerosis in male but not in the female cholesterol-fed rabbits.[4,5]

(*c*) *The Coronary Arteries.*—Katz et al. have observed that, with estrogen treatment, coronary atherosclerosis is prevented and may also regress in cholesterol-fed chickens.[6] Similarly, necropsy observations have demonstrated that male patients with prostatic cancer given prolonged estrogen treatment showed less coronary atherosclerosis than a similar nontreated group.[7] In the coronary arteries of rats, estrogens apparently exhibit a dual action; before the 60th day of administration of the hormone, coronary atherosclerosis is increased, while it is decreased afterward.[8] In the cholesterol-fed rabbit, on the other hand, estrogens do not affect coronary atherosclerosis.[9]

The mechanism through which estrogens may influence vascular structures is not known, and an indirect effect through induced changes in the blood lipid levels has been suggested. This blood effect, if present, is apparently not important, since women, although relatively protected from coronary atherosclerosis, may exhibit more aortic atherosclerosis than men,[10] thus pointing to other, still unknown, local mechanisms. Furthermore, since estrogens antagonize testicular function, the role played by the male gonads in relation to atherosclerosis may be questioned. In other words, are males more prone to coronary atherosclerosis because testicles are present or because of a lack of estrogens? The evidence at present is insufficient because although estrogens decrease coronary atherosclerosis in human beings [7] as well as in chickens [6] and in rats [8]

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* A recent report [21] shows that estradiol, but not dethylstilbestrol, may decrease aortic atherosclerosis of cholesterol-fed chickens; hence, a more precise definition of the word "estrogens" may be necessary in the future.

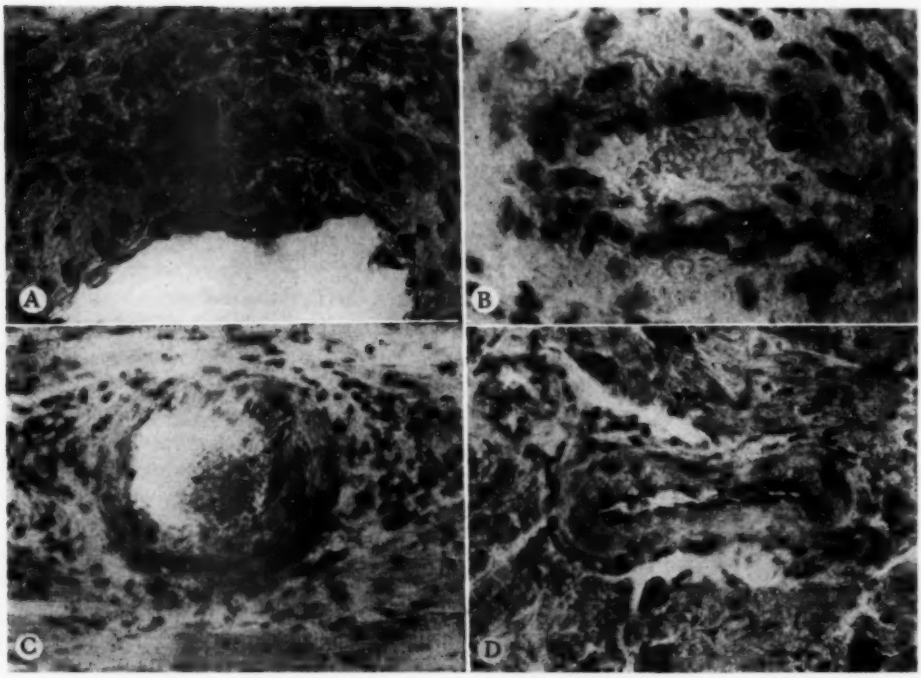

Fig. 1.—Coronary arteries of perinephritic oil-fed rats; hematoxylin and Sudan IV. *A*, Rat 2897. A proliferated intimal plague with sudanophilic cells; a few connective and lymph cells are seen at the adventitia (Grade 0.5+2.5, see Methods); reduced 33% from mag. × 450. *B*, Rat 2898. Proliferated endothelial cells loaded with sudanophilic granules; a few connective cells in the adventitia (Grade 0.5+2.5. see Methods); reduced 33% from mag. × 700. *C*, Rat 2611. Proliferated endothelium showing sudanophilic granules. The black spot on the right corresponds to lipid-stained material within proliferated cells. The media and the subintima are diffusedly stained with Sudan. A few connective and lymph cells are seen in the adventitia (Grade 0.5+2.5, see Methods); reduced 33% from mag. × 400. *D*, Rat 2576. Sudanophilic granules are seen within and in between the proliferated endothelial cells; few connective cells are present in the adventitia (Grade 0.5+2.0, see Methods); reduced 33% from mag. × 420.

castration decreases coronary lesions in male patients [11] but not in other animals.[8,12]

The conflicting results thus reported prompted us to study the effects of estradiol benzoate on the development of atherosclerosis in male rats. The following parameters have been studied: (*a*) spontaneous atherosclerosis, (*b*) experimentally induced atherosclerosis, (*c*) the development of vascular lesions in several territories, and (*d*) the influence of testicular atrophy upon the vascular effects.

Material and Methods

Male white William strain rats, between 15 and 24 months old and kept on a standard laboratory diet including bread, milk, meat, raw vegetables, and additional vitamins, were separated in three experimental groups: (*a*) 10 rats with an average weight of 234 gm. at the end of the experiment received 5μg. to 10μg. of α-estradiol benzoate intraperitoneally; (*b*) 40 rats with an average weight of 249 gm. in which unilateral cellophane perinephritis was performed under clean but not sterile technique; these animals also received 5 cc. of sunflower seed oil (I. N. 130) by gastric gavage; (*c*) 18 animals (average weight 179 gm.), as in (*b*), received in addition 5μg. to 10μg. of α-estradiol benzoate (procedures [*a*] through [*c*] were carried out daily except Sundays during periods up to 60 days). The control group of animals included 30 previously reported rats with an average weight of 326 gm.[13]

The method of study has been described previously [13] and will not be repeated. The only differences consisted in that the arterial sections were not counted, since several experiments showed

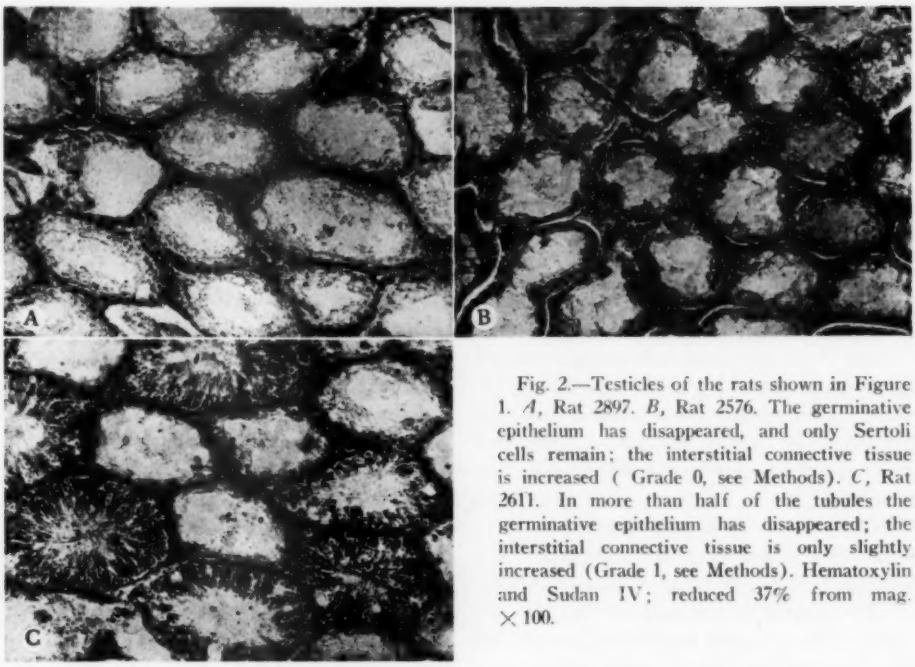

Fig. 2.—Testicles of the rats shown in Figure 1. *A*, Rat 2897. *B*, Rat 2576. The germinative epithelium has disappeared, and only Sertoli cells remain; the interstitial connective tissue is increased (Grade 0, see Methods). *C*, Rat 2611. In more than half of the tubules the germinative epithelium has disappeared; the interstitial connective tissue is only slightly increased (Grade 1, see Methods). Hematoxylin and Sudan IV; reduced 37% from mag. × 100.

us that for each rat we studied an average number of 20 lengthwise aortic sections, 170 coronary arteries in 60 cardiac sections, and 160 renal arteries in 60 kidney sections. Also the grading has been modified; in all arteries greater than 30μ and clearly showing the different wall structures lesions were graded as follows (tangential sections were disregarded): grade 0.5, initial endothelial and/or subendothelial infiltration of isolated Sudanophilic granules; Grade 1.0, medium-sized endothelial and/or subendothelial infiltration of Sudanophilic confluent granules; Grade 1.5, marked endothelial and/or subendothelial Sudanophilic infiltration, forming a total or partial obstruction; Grade 2.0, initial endothelial and/or subendothelial cellular proliferation of isolated cells; Grade 2.5, medium endothelial-and/or subendothelial-cell proliferation, forming small plaques; Grade 3.0, marked endothelial-and/or subendothelial-cell proliferation, forming partial or total obstructions with or without superimposed degenerative changes. In order to quantitate the results, each type of lesion in a given organ was multiplied by the following factor: ×1, if they numbered 1 to 5 different lesions as defined above; ×2, if they numbered 6 to 10; ×3, if they numbered 11 to 15, etc. By this procedure we tried to prevent computation of a single lesion as if it were more than one. When infiltrative and proliferative changes were present in a single lesion the highest grading was considered. All such lesions were then added in each organ and the results averaged for the entire group of rats. (In the previous report the arterial changes were averaged for each organ, but we feel now that by adding all lesions a more representative information is obtained; incidentally, previous results are not greatly altered because very few lesions were present in each organ.)

Additional techniques included study of the testicles in all animals and in 36 control rats: after removal the organs were weighed and fixed in 10% formaldehyde; one testicle was transversely cut and frozen, and four sections were stained with hematoxylin and Sudan IV. Testicular function was roughly graded as follows: 0, no spermatozoids were seen; 1, these cells were seen in less than half of the examined tubules; 2, spermatozoids were present in half of the examined tubules; 3, more than half of the tubules showed spermatozoids, and 4, all tubules showed spermatozoids. In the final interpretation testicles graded 0 through 1 were considered as atrophic and those with Grades 3 or 4, as normal.

Results

Blood Values (Tables 1 and 2).—In 9 estrogen-treated intact rats, blood cholesterol averaged 80 mg/100 ml. (range, 55 to 119);

TABLE 1.—*Laboratory and Autopsy Findings in the Four Groups of Rats* *

Group	No. Rats	Average Wt., Gm.	Average Heart Wt./Body Wt.×100	Average Testicle Wt./Body Wt.×100	Rats with Histologically Normal Testicles, %	Blood Urea, Mg./100 ml.	Blood Cholesterol, Mg./100 ml. [14]
Intact control [14]	30	326 (30)	0.32 (30)	0.83 (36)†	72 (36)†	36 (21)	95 (13)
Estradiol benzoate	10	234 (10)	0.37 (9)	0.86 (9)	77 (9)	59 (8)	80 (9)
Perinephritis	40	249 (34)	0.42 (32)	0.93 (27)	84 (26)	40 (28)	116 (23)
Perinephritis + estradiol benzoate	18	179 (17)	0.50 (17)	0.44 (17)	6 (17)	35 (10)	92 (6)

* No. of observations is within parentheses.
† Additional animals studied for the present paper.

in 23 perinephritic animals, 116 mg/100 ml. (range, 80 to 150), and in 6 estrogen-treated operated-on rats, 92 mg/100 ml. (range, 61-102). This should be compared with the control values of 95 mg/100 ml. obtained in 13 control rats, which shows that neither operation nor estrogen administration changed many blood cholesterol levels.

In 8 estrogen-treated rats blood urea averaged 59 mg/100 ml. (range, 43-71); in 28 operated-on animals, 40 mg/100 ml. (range, 23-60), and in 10 estrogen-treated operated-on rats, 35 mg/100 ml. (range, 10-65). In 21 animals of the control group the average blood urea was 36 mg/100 ml.,[14] which differed only from the estrogen-treated group of the present series.

Macroscopic Changes (Tables 1 and 2).—Heart Weight: The heart-body weight ×100 ratio was below 0.4 in all but one of the control animals, and similar values were seen in the estrogen-treated intact rats. In most of the operated-on animals the heart appeared enlarged, and the ratio was above 0.4 in 11 out of 32 rats not receiving estrogens and in 14 out of 17 receiving the hormone; this hypertrophy can probably be correlated with an elevated blood pressure induced by the perinephritis. Although it could appear that estrogens increased the incidence of enlarged heart in the operated-on rats, when the ratio was correlated with body weight (graph not shown) it was seen that both groups of animals behave similarly for comparable weights. Furthermore, when the heart-body weight ratio was plotted against the arterial intimal lesions (see below) it was apparent that no relationship existed between both.

Inflammatory Changes: As seen in most colonies of rats, suppurative lesions were common in this series. One estrogen-treated animal had small abscesses near the urinary bladder and the mesenterium, while the majority of the operated-on rats had differently sized abscesses around the intact or the operated kidney.

Testicles: When testicles were graded as described under Methods, histologically normal gonads were found in 26 out of the 36 control rats, in 7 out of 9 estrogen-treated intact animals, in 22 out of 26 operated-on rats, and in only 1 out of 17 operated-on hormone-receiving animals. It is apparent that a similar dosage of estrogens induced testicular atrophy more readily in operated-on than in intact rats, although the reason is not apparent. By plotting the testicular-

TABLE 2.—*Number of Animals Showing Microscopic Inflammatory Lesions in the Four Groups of Rats*

Group	Aorta		Aortic Branches		Coronary Arteries			Renal Arteries		
	Adventitial	Intravascular	Adventitial	Intravascular	Parenchymatous	Adventitial	Intravascular	Parenchymatous	Adventitial	Intravascular
Intact control [14]	17	4	8	4		21	2		30	0
Estradiol benzoate	6	1	8	4	10	7	0	5	10	0
Perinephritis	30	2	25	4	38	38	3	32	40	6
Perinephritis + estradiol benzoate	18	0	13	2	12	18	1	14	18	2

body weight ratio against weight of the animals it became apparent that the ratio decreased with increasing weight of the animals. In the control rats this ratio averaged 0.83 (range, 0.16-1.38); in the estrogen-treated intact animals, 0.86 (range, 0.29-1.01); in the operated-on animals, 0.93 (range, 0.38-1.38), and in the operated-on hormone-receiving rats, 0.44 (range, 0.20-0.87). The histological appearance could be predicted from the testicular-body weight ratio, since with only two exceptions spermatozoids were not present if the ratio was below 0.70 and were invariably present in normal proportions whenever it was above 0.80.

Microscopic Changes (Tables 2 through 4).—(1) Parenchymatous Inflammation (Table 2): Microscopic evidence of inflammation was seen in practically all animals. Lymphocytic with or without monocytic infiltration was observed in the myocardium of all estrogen-treated intact rats, in 38 operated-on animals, and in 12 operated-on hormone-receiving rats. A similar type of myocarditis was also seen in most of the control animals.[14]

Nonspecific lymphocytic with or without coincident monocytic infiltration was seen in most of the non-operated-on kidneys, as was also true for the control group.[13] In addition, the operated-on kidney showed very commonly the presence of abscesses around and within the organ.

(2) Arterial Findings: Normal arteries of rats have been described at length elsewhere[14] and shortly summarized in this the ARCHIVES.[13] In the present series, inflammatory and degenerative changes were frequently seen.

(*a*) Inflammatory changes. Cellular infiltration of lymphocytes, histocytes, and polynuclear leukocytes was a common occurrence in the studied arteries, although no changes similar to periarteritis nodosa were seen. The incidence of adventitial infiltration and its extension into the arterial wall is depicted in Table 2. No relationship was found between the incidence of inflammation and the arterial degenerative changes to be discussed below.

(*b*) Medial degenerative changes (Table 3). Lipid infiltration. In the coronary arteries of only two (20%) estrogen-treated intact rats lipid infiltration of the media was found. This must be compared with the following findings in the control group: five animals (17%) showed this medial change in the immediate aortic branches; seven (23%), in the coronary arteries, and six (20%), in the renal arteries. Consequently the incidence was reduced in all arteries except the coronary.

In the operated-on group findings were as follows: 14 rats (35%) showed medial lipid infiltration in the aortic collaterals; 20 (50%), in the coronary arteries, and 25 (62%), in the renal vessels. In the operated-on estrogen-treated rats the incidence was similar: in 5 animals (27%), in the aortic collateral branches; in 6 (30%), in the coronary, and in 10 (55%), in the renal arteries.

Calcification. Basophilic plaques extending into the subintima were seen in six animals (20%) of the control group and in

TABLE 3.—*Number of Animals Showing Medial Degenerative Lesions in the Four Groups of Rats* *

Group	Aorta		Aortic Branches		Coronary Arteries		Renal Arteries	
	Medial Lipid Infiltration	Calcification	Medial Lipid Infiltration	Calcification	Medial Lipid Infiltration	Calcification	Medial Lipid Infiltration	Calcification
Intact control [13]	0	0	5 (12)	6 (12)	7 (9)	0	6 (9)	0
Estradiol benzoate	0	0	0	0	2 (5)	0	0	0
Perinephritis	0	0	14 (83)	4 (15)	20 (205)	0	25 (445)	0
Perinephritis + estradiol benzoate	0	0	5 (22)	0	6 (90)	2 (50)	10 (72)	3 (7)

* No. sections showing lesions is within parentheses.

TABLE 4.—*Incidence of Intimal Degenerative Lesions in the Four Groups of Rats*

Group	Atherosclerosis											
	Aorta			Aortic Branches			Coronaries			Renal		
	No. Rats	%	Grade	No. Rats	%	Grade	No. Rats	%	Grade	No. Rats	%	Grade
Intact control	1	3	0.08	4	13	0.32	10	33	0.48	3	10	0.10
Estradiol benzoate	0	0	0	0	0	0	0	0	0	0	0	0
Perinephritis	2	5	0.10	8	20	0.56	15	37	1.98	4	10	0.27
Perinephritis + estradiol benzoate	0	0	0	0	0	0	5	27	1.94	3	16	0.41

none of the estrogen-treated rats. In the operated-on groups calcification was seen in the aortic collateral branches of four (10%) perinephritic rats and in the coronary and the renal arteries of two (11%) operated-on estrogen-receiving rats. Intimal degenerative changes (Tables 3 and 4). Lipid infiltration (atheromatosis). In none of the estrogen-treated intact animals was intimal infiltration seen. Since this lesion was present in 16 out of 30 control rats,[14] it can be concluded that estrogens decreased the incidence of the minimal atheromatous lesions spontaneously present in this species.

In the operated-on group 1 rat showed intimal lipid infiltration of one aortic section; 7 (18%), in 29 sections of the aortic collateral arteries; 14 (35%), in 140 sections of the coronary arteries, and 4 (10%), in 18 sections of the renal vessels. It is readily apparent that perinephritis increased the incidence of infiltrative lesions when compared with the control group. In the operated-on estrogen-treated animals the lesions were somewhat decreased when compared with the operated-on group; none showed these lesions in the aorta or in the aortic collateral branches, 3 (16%) showed them in 37 sections of the coronary arteries, and 3 (16%), in 9 sections of the renal vessels. Although the incidence of the coronary infiltrative lesions appeared decreased, when judged by its extension and/or proliferation (see below) a similar involvement was present in both series.

Intimal proliferation (atherosclerosis). In none of the intact animals receiving estrogens was intimal proliferation seen; in the control rats atherosclerosis was present in only four arterial sections of one animal; consequently, no conclusions can be drawn from these results.

In the operated-on group, 2 animals (5%) showed atherosclerosis in 4 sections of the aorta; 4 (10%), in 8 sections of the aortic collateral branches; 8 (20%), in 86 sections of the coronary arteries, and 2 (5%), in 2 sections of the kidney vessels. In the operated-on estrogen-treated group, intimal proliferation was not seen in the aorta or in its immediate collateral branches but was found in 38 coronary sections of four animals (22%) and in 3 renal sections of two animals (11%). The extension of the lesions can be seen in Table 4.

When the lesions were graded and averaged for the different groups of rats (Table 4) the following results were obtained in the aorta, its immediate collateral branches, and in the coronary and the renal arteries, respectively (percentages of animals is given between parentheses): estrogen-treated group, 0,0,0,0 (which should be compared with 0.08 [3%], 0.23 [13%], 0.48 [33%], and 0.10 [10%] in the control animals); perinephritic rats, 0.10 (5%), 0.56 (20%), 1.98 (37%), and 0.27 (10%); perinephritic estrogen-treated animals, 0,0,1.94 (27%), and 0.41 (16%). Here it is seen again that estrogens abolished spontaneous arterial lesions in all territories, while the hormone did not reduce its extent in the coronary and the renal vessels of the operated-on animals.

Relationship between intimal degenerative changes and testicular atrophy. Graded intimal degenerative histologically changes when plotted against the testicular-body weight ratio showed that no relationship existed between both parameters. Furthermore, while the incidence of histologically

normal testicles was 72% in the control group, it was similarly 77% in the estrogen-treated group where atherosclerosis was absent; contrariwise, while 84% of the perinephritic rats had normal testicles, in only 6% of the operated-on estrogen-treated animals was this finding present in spite that atherosclerosis was not prevented in the coronary or in the renal arteries of this group.

Comment

Rats have been shown to be suitable animals for the study of atherosclerosis, and spontaneous [13] as well as experimentally induced atherosclerotic lesions have been reported in this species by several authors, including ourselves.[15-18] In the present paper it is demonstrated that oil-fed perinephritic rats have more pronounced arterial lesions than intact control animals; similar conclusions based on a somewhat different method have also been reached by Wissler et al.[17] The mechanisms involved remain to be elucidated, but it must be pointed out that contrary to what happens in other species, no relationship was found between increased heart weight, probably secondary to arterial hypertension, and the development of atherosclerosis in the rat, a conclusion already advanced by Wissler et al.[17]

Sexual glands have been clinically related to the development of atherosclerosis, as evidenced by the predominantly male population of patients with coronary disease as well as by the protective effects of estrogens or castration in men.[7,22] Experimental findings supporting this relationship have been presented by Pick et al., showing that estrogenic substances greatly influence coronary atherosclerosis in chickens.[6] Moskowitz et al.[8] also showed that in the rat a similar protection is effected when a high dosage of estrogenic substances is given, and the same effect was seen in our experiments conducted in intact rats when treated with a considerably lower dosage. Furthermore, in the present experiment this protection was shown to affect other vessels, namely, the aorta,† the immediate aortic branches, and the renal arteries of the same group of animals, as well as to the aorta and nearby branches in oil-fed perinephritic rats.

In contrast, estradiol benzoate did not prevent atherosclerosis of the coronary or the renal arteries in our oil-fed perinephritic animals. This finding which appears to contradict Katz' and Moskowitz' results can not be totally explained at the present time, but the following factors must be considered: (*a*) Species differences; the arteries of rats behave differently from those of the chicken, for instance, in connection with estrogens which can increase aortic atherosclerosis in birds,[2] while no such increase was produced in the present series; a similar lack of effect is also seen in the rabbit.[3] Hypertension, too, does not increase the incidence of atherosclerosis in the rat, while the contrary is true in other species.[19] (*b*) Time of observation too short; it has been reported that early during estrogen administration an increased incidence of coronary arterial degenerative changes occurs [5]; as we limited this observation to 60 days, our animals could be within the increased incidence time of Moskowitz et al.[8] Nevertheless, in the present series we observed not an increased but a decreased incidence of atherosclerosis in other vascular territories. (*c*) Dosage too low; the dosage of estradiol benzoate found to decrease coronary atherosclerosis after 90 days in nephritic rats given a high-fat diet was 0.333 mg. per rat per day.[8] We used only 5μg. to 10μg. of α-estradiol benzoate trying to separate the arterial effects from those exercised upon the testicles; perhaps higher dosages could have had the same effects as those obtained by Moskowitz et al.[8]

From the present series as well as from the experience of others it is apparent that estrogens can reduce atherosclerosis in different territories of several species. The

† It must be pointed out that lesions in the aorta are difficult to produce; consequently, although the estrogen-treated animals did not show aortic lesions, the difference with the nontreated animals was small.

mechanism of this action is not clear. A reversed plasma cholesterol-phospholipid ratio toward normal has been incriminated,[20] but other mechanisms may also be involved. The injection of estrogens in male animals, for instance, has an injurious effect upon the testicles which apparently is mediated through the hypophysis, since it can be prevented with gonadotropic hormones[21] (a direct action of estrogens upon testicular structures has also been postulated[22]). Although castration in man can apparently reduce the incidence of atherosclerosis,[11] it has not been established whether or not the protective action of estrogens in males is mediated through an injurious effect upon the testicles. Our experiments show, we believe, that testicular atrophy must not necessarily be induced by estrogens in order to protect the animals from the development of atherosclerosis. This was confirmed by the fact that the incidence of testicular atrophy was not increased in the estrogen-treated group which was completely free from intimal lesions; contrariwise, the incidence of coronary and renal lesions was not decreased in the perinephritic animals with testicular atrophy. Similar results have been reported in castrated rats.[8] As a consequence, although castration reduces atherosclerosis in males and although estrogens can produce testicular atrophy, it seems possible that these hormones can influence arterial lesions through another, as yet unknown, mechanism.

Summary

Five to 10 micrograms of α-estradiol benzoate was given intraperitoneally during 60 days to intact and to oil-fed perinephritic male rats. The aorta, the nearby aortic collaterals, and the coronary and renal arteries were studied histologically. By comparing with corresponding control groups it was found that the estrogenic substance prevented the appearance of atherosclerotic lesions in all studied vascular territories of the intact rats, while a similar action was apparently seen only in the aorta and nearby branches of the operated-on animals. It is suggested that such a lack of influence upon coronary and renal atherosclerosis could be due to a lower dosage and/or a shorter time of observation. The present studies, by separating vascular from testicular effects, show that the arterial protection afforded by estrogens is not mediated through an induced atrophy of the testicles.

Dr. Eugenia H. Ramos performed the chemical determinations of the present study; Prof. Blas Moia helped during the completion of these experiments. Prof. E. Braun Menéndez corrected the manuscript of the present paper.

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Metastatic Carcinomatous Cirrhosis of the Liver

Report of a Case in Which Death Followed Hemorrhage from Esophageal Varices and Hepatic Coma

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The title in this instance designates a severe and diffuse form of hepatic fibrosis that was caused by metastatic carcinomatosis of the liver. The gross anatomical and clinical characteristics of advanced portal cirrhosis, including portal hypertension with esophageal varices, were present.

Increased resistance to the flow of blood in the portal vein is most frequently the result of the complex alterations within the liver indicated as "cirrhosis." This state is usually, but not always, characterized by easily recognizable functional derangements. Less often extrahepatic portal vein thrombosis causes the obstruction to flow; commonly there are no abnormalities in the results of the usual liver-function tests. Portal hypertension and its concomitants, esophagogastric varices, may also follow metastatic carcinoma in the liver. This last sequence is rarely recognized clinically. The essential feature of the present case was the association of massive metastasis to the liver of a scirrhous carcinoma of the breast, with a clinical picture resembling that of hepatic necrosis and cirrhosis. The manifestations of jaundice, spider angiomata, dilated superficial abdominal veins, ascites, hepatosplenomegaly, bleeding esophageal varices, and hepatic coma were impressive to the clinicians. Furthermore, at the autopsy table gross examination revealed hepatic fibrosis and failed to disclose evidence of metastasis to the liver; the condition was recognized only after microscopic examination.

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Report of Case

A 42-year-old white housewife and schoolteacher was admitted to the New England Center Hospital on April 9, 1956. Her chief complaint was "stomach distention." A left radical mastectomy had been done in May, 1955; she had received three blood transfusions. The histopathological report of the excised breast tumor was "scirrhous carcinoma," with vascular invasion of the axillary contents by the tumor. There was no evidence of metastasis to 24 lymph nodes examined. She made an uneventful recovery and was well until January, 1956, when she became fatigued and developed a headache rather abruptly. These symptoms persisted; she continued to teach, however, until February, when anorexia, nausea, vomiting, weight loss, and abdominal distention caused her to be admitted to another hospital for 10 days. One month prior to admission her sclerae became yellow; her urine, dark, and she had continued nausea, vomiting, and considerable belching. Her condition progressively worsened before admission.

There was no history of alcoholism, recent abdominal surgery, contact with patients with infectious hepatitis, parenteral injections, ingestion of hepatotoxins or use of such things as cleaning fluids, contacts with rats, or residence in rat-infested areas.

Past History.—A cæsarian section was done in 1944. From age 9 to 14 years she had "growing pains," which occasionally necessitated absence from school. One year prior to admission she had "virus pneumonia."

Family History.—Her mother had died of "cancer of the cervix" at age 30 years. Her grandfather died of cancer of the lung.

Physical Examination.—Examination disclosed a chronically ill lethargic white woman with abdom-

inal distention. She was deeply jaundiced. There were spider angiomata of the skin over the left wrist, right cheek, and right shoulder. There was a healed left anterior chest scar. The left breast was absent. The right breast contained no abnormal masses. There was a Grade 2 pulmonic systolic murmur. The left lung was normal. The lower half of the right lung field posteriorly was dull on percussion, with dullness rising into the axilla. There were increased tactile fremitus, increased transmission of the whispered voice, bronchovesicular breathing, and egophony without rales over this area. The abdomen was distended with fluid. There was a visible venous pattern over the abdominal wall. The lower edge of the liver was felt 8 cm. below the right costal margin; it was firm and without palpable nodules. The spleen was palpable 2 cm. below the left costal margin. There was 2+ pitting edema of the lower extremities.

Laboratory Studies.—Laboratory studies revealed a normal hemoglobin value, hematocrit reading, white blood cell count, and differential count. The urinalysis disclosed a specific gravity of 1.023, absent sugar, and 0.005 gm/100 ml. of albumin. Bile was present. The urinary sediment contained no casts, but there were showers of pus cells, on occasion 30 to 60 per high power field. Prothrombin time was 22 seconds, 14% of the normal content. The erythrocyte sedimentation rate was normal. Concentrations of sodium, potassium, chloride, and bicarbonate in the serum were normal. The total serum cholesterol was 342 mg/100 ml. Of the total cholesterol 16% was esterified. Total serum bilirubin was 11 mg/100 ml., with 7.3 mg. direct reading and 3.7 mg. indirect. The serum contained 2.3 gm/100 ml. of albumin and 3.8 gm/100 ml. of globulin. Thymol turbidity (serum) was 4 units. The serum Hinton and Kahn tests were negative. The stools were grossly acholic and guaiac-negative.

Lateral and posteroanterior views of the chest showed both diaphragms and costophrenic angles to be clear. The heart was normal in size and shape. No pulmonary infiltrations were noted. The bony thorax and the mediastinum were normal. A metastatic bone series disclosed no evidence of a metastatic lesion.

Course in the Hospital.—The patient remained a problem in diagnosis, since it was difficult to determine whether she had severe "hepatitis," metastatic involvement of the liver, or both. She had persistent anorexia. She received daily infusions of dextrose supplemented with all the known available water-soluble vitamins. She also received infusions of salt-poor human serum albumin in an effort to increase the concentration of serum albumin. Daily injections of vitamin K were given. She showed slight improvement during the first five days, but the remainder of the two-and-one-half-week hospital course was gradually downhill. She continued anorexic and took little food by mouth. On the 10th hospital day she vomited blood. A fetor was present, and she became progressively drowsy. A Sengstaken tube was inserted, and multiple blood transfusions were given. There were oliguria, rising BUN, and mild hyperkalemia; the urinary sediment contained granular and hyaline casts, red blood cells, and renal tubular cells. On the 14th day, after further hematemesis, the blood pressure dropped to 90/60. Further blood transfusions were given, and other measures were instituted to combat shock. She became weaker and totally unresponsive. The serum bilirubin gradually rose from 11 mg/100 ml. to 35 mg/100 ml. on the day prior to death. On the 16th hospital day she died.

Autopsy Findings

Gross Examination

The skin and sclerae were markedly icteric. There was pitting edema of the lower extremities and sacral area. The right breast was grossly normal. There was a well-healed left radical mastectomy scar. No recurrent tumor could be seen in the area, and no enlarged axillary, cervical, or inguinal nodes could be found. The abdomen was distended, and the peritoneal cavity contained 5500 cc. of slightly turbid sterile yellowish fluid; the surfaces were everywhere smooth and free of tumor nodules.

The liver was only slightly enlarged but weighed 2100 gm. The surface was rather uniformly nodular. Small raised greenish-brown areas of liver parenchyma alternated with shallow communicating grayish scars (Fig. 1). The organ had an unusually firm rubbery consistency and could be cut only with difficulty. The pattern of the cut surface was that of greenish-brown irregular islands of recognizable liver tissue, fairly evenly dispersed in a sea of whitish-gray dense fibrous tissue. This same picture was seen on all sections (Fig. 2). There were no discrete tumor nodules. The portal vein and its tributaries and the extrahepatic bile ducts were patent.

In the lower third of the esophagus there were numerous and very prominent varices, with two small ulcerations in the overlying mucosa. No lacerations indicative of the Mallory-Weiss syndrome could be found in the mucosa. The stomach and small and large bowel, free of ulcers or tumor, were filled with large amounts of dark red thick blood.

The remainder of the autopsy can be reviewed very briefly. The spleen weighed 220 gm., was soft and reddish-brown, and did not resemble

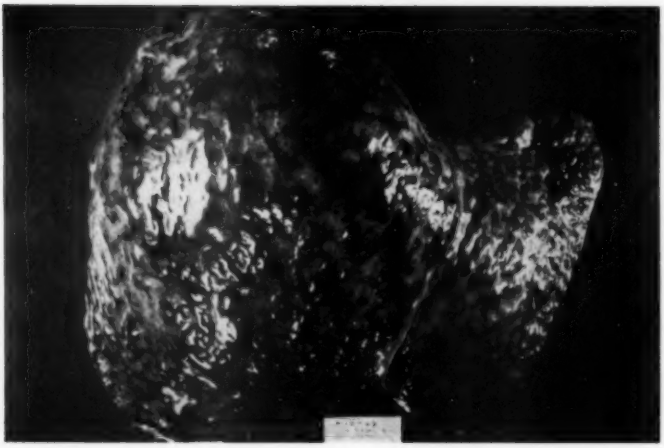

Fig. 1.—The external surface of the liver, showing the diffuse coarsely nodular pattern and the shallow communicating scars.

the spleen of chronic passive congestion. The gall bladder and pancreas appeared normal. The kidneys, of average size, were intensely bile-stained. There were petechial hemorrhages in the pelves, ureters, bladder, and rectum. The adrenals, smaller than usual, showed lipid depletion of the cortex. The internal genital organs and lymph nodes from the hilus of the liver, mesentery, and para-aortal areas were grossly normal. The thyroid was normal. The right pleural cavity contained 200 cc. of thin yellowish fluid; the left, 100 cc. There were no tumor nodules on the parietal pleura. The heart was small and flabby, with petechial hemorrhages on the epicardial and endocardial surfaces. The brain and meninges appeared normal.

Neoplastic tissue was first recognized in the lungs, both of which showed a fine pleural studding, with minute whitish nodules and thready branching lines, 1 or 2 mm. in diameter. There were no nodules in the parenchyma. Tumor was again encountered in the hilar and mediastinal lymph nodes, in the nodes along the left internal mammary artery, and finally in the vertebral bodies of the lower thoracic and lumbar spine.

On the basis of these findings the following gross anatomic diagnoses were made: (1) advanced cirrhosis of the liver, of undetermined type, with ulcerated esophageal varices and massive hemorrhage into the alimentary tract; (2) metastatic carcinomatosis of lungs and bone marrow and lymph nodes of mediastinum, pulmonary hilus, and internal mammary chains.

Any pathogenetic connection between these two main diseases, although strongly suspected, could not be verified by the gross findings at the autopsy table.

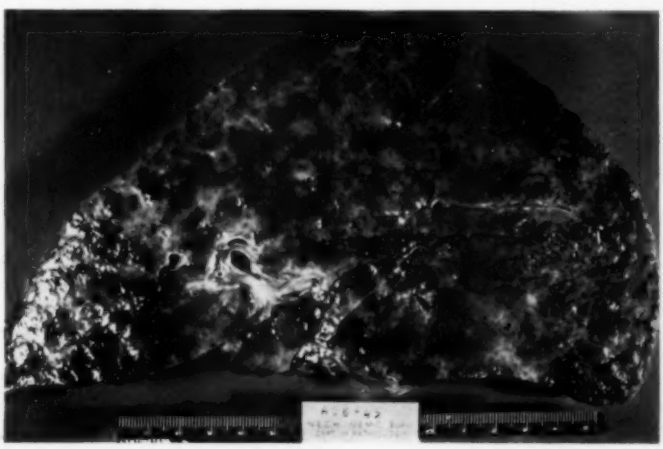

Fig. 2.—The cut surface. Note the darker areas of remaining liver parenchyma interlocking with the lighter areas of dense fibrous tissue.

Fig. 3.—A typical low-power field showing the extensive fibrosis with widespread destruction of liver lobules. Reduced 40% from mag. × 45.

Microscopic Examination

The most striking changes were found in the liver. This entire organ was diffusely infiltrated by tumor tissue that directly and indirectly through interference with the circulation had destroyed much of the liver parenchyma and replaced it with coarse communicating partitions of fibrous tissue (Fig. 3). Most of the normal lobular pattern was completely lost, but here and there portal triads were still discernible. This picture was complicated by recent hemorrhage and bile stasis. The tumor was composed of small epithelial cells occurring singly, in small clumps, and in cords (Fig. 4). The cells were disseminated freely and appeared to encroach upon the lobules from the portal areas (Fig. 5). At times the sinuses were infiltrated; at other times there was direct invasion into the liver trabeculae. Branches of the portal vein as well as the sinuses became obliterated, and the same reaction in the portal areas led to considerable intrahepatic bile stasis.

Three major sequential histologic changes were taking place in the liver: first, an infiltration of tumor cells into the liver cords, producing lysis and disappearance of liver cells; second, a gradual pyknosis and necrosis of tumor cells, with widespread re-

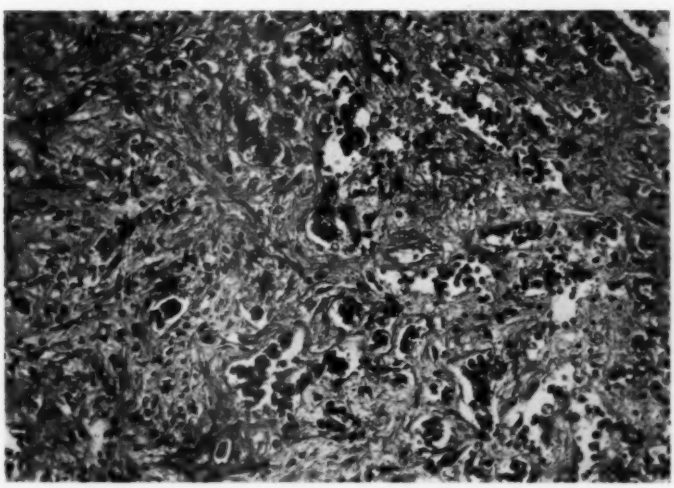

Fig. 4.—This field was selected to show the arrangement of tumor cells in clusters and cords, embedded in fibrous tissue. Reduced 35% from mag. × 260.

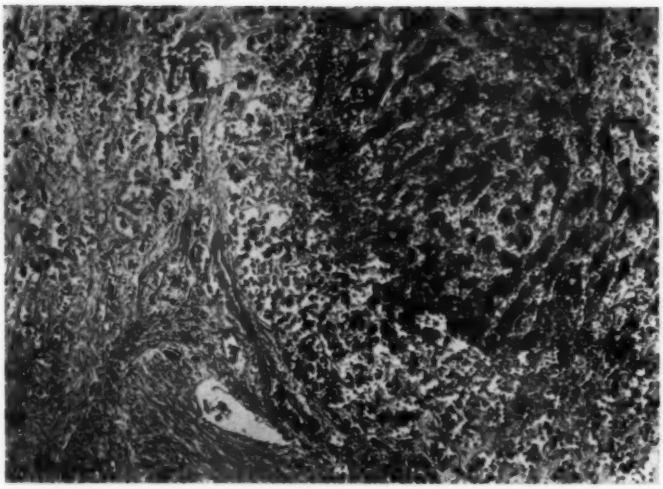

Fig. 5.—Tumor cells are seen radiating out from the portal areas and encroaching on the periphery of the liver lobules. Reduced 30% from mag. × 120.

placement by fibrous tissue in the form of a relatively acellular sclerosing process, and third, a most recent change, characterized by partial necrosis of remaining liver parenchyma, bile stasis, and hemorrhage. The over-all picture was one of diffuse hepatic fibrosis or severe "cirrhosis," with an extensive destruction of liver parenchyma and a nearly complete interruption in the normal vascular bed.

The microscopic examination of the lungs revealed widespread lymphangitic carcinomatosis, involving pleural and parenchymal lymphatics. No tumor cells could be seen in the blood vessels. The mediastinal, internal mammary, and pulmonary hilar lymph nodes were partially replaced by metastatic tumor; here the tumor initiated a marked desmoplastic reaction, creating the pattern of a scirrhous carcinoma. Nests of tumor cells were also seen in the subserosal lymphatics of the gallbladder, in the lymphatics of the pancreas and periadrenal fat, and in the bone marrow. In all these locations the neoplastic cells were identical morphologically with those encountered in the liver.

The right breast was free of any primary or metastatic tumor. However, other findings after histological examination were varices and acute ulcerations of the esophagus, pulp hyperplasia and erythroid metaplasia of the spleen, renal tubular necrosis and edema, and spotty regressive changes in the brain.

Comment

The unusual interest and importance of this case lies in the fact that a metastatic tumor had produced a disease in the liver that anatomically and clinically mimicked advanced portal cirrhosis.

Anatomically the dominant feature was a diffuse fibrosis or "cirrhosis" of the liver, initiated by a sclerosing metastatic carcinoma. Portal hypertension, ascites, edema, massive bleeding from esophageal varices, and ultimately liver failure followed as the "cirrhotic" process evolved.

The possibility of an underlying cirrhosis upon which metastatic carcinomatosis was superimposed was considered but rather convincingly excluded. The basis for such an exclusion was the absolute lack of any suggestive data in the clinical history; this was corroborated by the histological observation that in the interlobular and portal spaces of the preserved islands of liver tissue there was no fibrosis or cirrhosis except in areas where the tumor cells were intrinsically involved.

In the absence of another tumor at the time of autopsy the scirrhous breast carcinoma, surgically resected 11 months pre-

viously, presumably was the primary lesion. A comparative study of the histological characteristics of the tumor in the surgical and autopsy specimens confirmed this conclusion. In addition to the morphologic identity of the tumors, an invasion of lymphatics was seen in the surgical specimen that was similar to the pattern of lymphatic dissemination found at autopsy.

In reviewing the various anatomical patterns of metastatic carcinoma to the liver one recognizes that in addition to the well-known and most frequent nodular variety other less common patterns exist. Occasionally metastatic cancer cells diffusely infiltrate the liver. In most of the examples in the literature gross tumor nodules were also present. Very rarely the condition was recognized only on microscopic examination; the cases of Craciun et al.,[1] Oertel,[2] and Botha and Lennox [3] were representative. In our case also it was not possible to recognize the condition with the unaided eye. Watson [4] recently reemphasized another rare variety, in which there was a diffuse intrasinusoidal infiltration of neoplastic cells, either with or without small discrete tumor nodules. In two of his five cases the presence of enlarged and tortuous veins on the abdominal wall were thought to indicate portal vein obstruction; no esophageal varices were recognized.

A fourth variety, by far the rarest, exemplified by our case, was that in which the diffuse infiltration by tumor cells—intrasinusoidal, perisinusoidal, or intratrabecular—was accompanied by an extensive scirrhous reaction, leading to a sequence of clinical and anatomic changes that justified the term "metastatic carcinomatous cirrhosis."

In our perusal of the literature we were able to find only one reported case of metastatic carcinomatosis of the liver producing a "cirrhosis" similar to that exhibited by our patient. The case, reported by Craciun, Aslan, and Caffé,[1] occurred in a female who 14 months previously had also undergone a radical mastectomy for a scirrhous carcinoma of the breast. The liver weighed only 1200 gm. The term used by the authors was "cirrhose atrophique néoplasique secondaire." The histological and clinical characteristics presented bear striking and interesting similarities to those of our case.

There is no clear reason why metastatic carcinoma to the liver should most often assume the nodular form, at times the diffuse infiltrative form, and rarely the form of "carcinomatous cirrhosis." To explain the hepatic fibrosis in their case Craciun et al. felt that the metastatic cells had retained the power to stimulate overgrowth of fibrous connective tissue that had characterized the primary scirrhous carcinoma. There is no conclusive evidence to indicate that a particular vascular route, venous, arterial, or lymphatic, would play a role in determining the anatomic pattern of metastases.

Little attention has been devoted to the role of hepatic metastasis in the production of portal-vein obstruction. Several textbooks dealing mainly with the liver [5-9] fail to mention metastatic carcinoma in the liver as a cause of esophageal varices. Esophageal varices associated with massive tumor metastasis to the liver have been described by Weinberg [10] in instances of primary tumors in the colon, adrenal cortex, stomach, and kidney. Duff,[11] in reviewing 16 cases of carcinoma of the pancreas involving the body and tail, encountered 2 who had hematemesis due to bleeding varices following portal obstruction caused by neoplastic deposits in the liver. In four other cases this was likely, but the bleeding points could not be found. Ruprecht and Kinney [12] reported four additional cases; three were adenocarcinoma of the large bowel, and the fourth was a bronchogenic carcinoma. In these four cases the obstruction to the flow of portal blood was attributed to the extensive metastases to the liver. In none of these instances was there any evidence of cirrhosis, nor were there thrombosis or extrahepatic compression of the portal trunk or any of its tributaries. The varices were asymptomatic

in one case; in the others there were multiple episodes of bleeding, and two of the patients died following massive hematemesis. Most of the hemorrhages occurred during the last few weeks of life, but in one case the first hematemesis occurred one year after the primary growth was removed from the colon and two years before death.

Our patient demonstrated most of the common clinical manifestations of severe "parenchymatous" liver disease, including jaundice, cutaneous spiders, progressive hepatic failure, and even the sequelae of portal obstruction. It has been our experience that these phenomena, with the exception of portal hypertension, are not uncommon in patients with metastatic cancer to the liver. The complications arising from portal hypertension added to the diagnostic difficulties; these last were finally solved only by microscopic examination of the liver.

Summary

A 42-year-old woman developed severe liver disease less than a year after a radical mastectomy for scirrhous carcinoma. The picture was characterized by jaundice, cutaneous spiders, hepatosplenomegaly, ascites, and laboratory evidence of marked functional disturbances. Bleeding from esophageal varices and coma were late manifestations. At autopsy the abnormality in the liver grossly seemed to be cirrhosis. It was recognizable only microscopically that widespread infiltration with tumor had been followed by diffuse hepatic necrosis and fibrosis. The observed portal hypertension could have resulted from either the primary or secondary abnormalities or both. The designation "metastatic carcinomatous cirrhosis" in this, the second such case in the literature, seems appropriate.

Dr. H. E. MacMahon assisted in the interpretation and presentation of the findings described in this paper.

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Gasteropagus in a Triplet Pregnancy

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Development of conjoined twins is uncommon, and their occurrence in a triplet pregnancy has not been described previously. The present report deals with an anatomical account of male triplet fetuses, two of which were conjoined abdominally.

Report of a Case

The mother was a 23-year-old white housewife who had been well except for the common diseases of childhood. Menarche began at 12 years of age, occurred at 28 to 30-day intervals, and was of 3 to 4 days' duration. Menstrual flow was moderate and without discomfort. For the past three to four months her periods had become heavier. The patient had enjoyed good health except for moderate hypothyroidism, which was controlled by administration of thyroid extract (½ grain daily). At 19 years of age she had a normal pregnancy terminated by an uneventful delivery and birth of a normal boy. One year later a second uncomplicated pregnancy culminated in delivery of a normal girl. Both children are living and well. The family history revealed no anomalies or multiple births in the mother's family. The paternal grandmother, however, was a twin, and the paternal grandfather had twin male siblings.

The last normal menstruation, on Jan. 20, 1956, occurred six weeks before the third pregnancy was diagnosed. Shortly thereafter painless irregular uterine bleeding began and continued through the remainder of the pregnancy. This hemorrhage was very slight, as a rule consisting of a pinkish vaginal discharge lasting for a few hours to one day. On May 7, 1956, severe uterine cramps began suddenly and were accompanied by progressively severe uterine bleeding. Pelvic examination revealed a patent cervix, with a fetal part protruding through the external os. The size of the uterus was consistent with that of a 3½- to 4-month pregnancy. Within a short time the patient was

delivered of three small fetuses spontaneously. Clotted blood passed from the vagina contained a fragmented hemorrhagic placenta measuring 6.0 cm. in its widest diameter. Placental membranes were absent.

Gross Description

Examination of the triplets (Figure) revealed a single fetus and conjoined twins. The single fetus measured 2.7 cm. in length and appeared normal and well formed. Each of the conjoined twin fetuses measured 2.5 cm. in length. They were symmetrical, faced each other, and were joined in an area extending from the xiphoid to the lower abdomen. There was considerably maceration of some portions of the skin, with fragmentation of the necks and of the superficial portions of the abdominal tissues uniting the twins. There were no demonstrable anomalies of the heads or extremities. Each fetus had a small genital tubercle. No umbilical cord segments remained attached. The conjoined twin fetuses were attached by a tube continuous with their abdominal walls, extending from the level of the lower sternums to the pubes. Folds of skin extended from the inferior surface of the joining tube to the upper portions of the thighs. The unjoined portions of the bodies were separated approximately 3.0 mm.

Examination under the dissecting microscope showed that the connecting tube contained an extension of the peritoneal space of each fetus. In the midportion of the tube a peritoneal layer divided the tubal space. The liver of each fetus was separate. Extending between the twin fetuses were two segments of gastroenteric tract, an upper larger one believed to be stomach, and a lower one representing a portion of the small intestine. The

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small size of the fetuses precluded further tracing of the gastroenteric connection between the two individuals. Each fetus had a segment of small bowel of normal length attached posteriorly by a mesentery and a colon in the normal position.

The thoracic viscera were grossly normal.

Inspection of the pelves of the conjoined twin fetuses and of the single fetus gave identical findings. Uteruses, uterine tubes, or vaginas could not be identified. In each lateral wall of each pelvis was a gonad measuring 1.0 mm. in its widest diameter. Attached to one margin of each gonad was a slender cord extending toward the inguinal region.

Microscopic Examination

The six gonads of the three fetuses had similar histologic appearances. The bulk of the tissue, presumably testis, consisted mainly of undifferentiated mesenchymal cells, having oval or round nuclei and relatively scanty cytoplasm. Some of the smaller cells were in small groups or elongated cords. Other larger undifferentiated cells had oval or round nuclei and more abundant cytoplasm. Immediately adjacent to the main gonadal stucture was a smaller ovoid mass, consisting of similar cells arranged in distinct groups forming small tubules. This structure was identified as epididymis.

Serial sections through the abdominal and thoracic segments of the conjoined twin fetuses showed normal thoracic viscera. The esophagus of each fetus was traced to a large epithelial-lined structure just beneath the liver, identified as stomach. Extreme postmortem degeneration of the mucosa precluded further identification. Extending between the two abdominal cavities of the two fetuses was a similar enteric tube, presumably small intestine. Connections of small intestine to the structure designated as stomach could not be demonstrated.

Serial sections of the pelvic segment of each twin fetus revealed normal arrangements of ureters and bladder. A urethra could be traced through the genital tubercle of each fetus.

Some portions of the epidermis were well preserved. No sex chromatin could be identified in the squamous epithelial cells.

Comment

A recently published statistical study of multiple births in the United States from 1933 to 1950 indicated that twins occur in the ratio of 1:92 deliveries; triplets, in a ratio of 1:9,600, and quadruplets, in the ratio of 1:657,000.[1] The occurrence of twins varies in different races. In Japan the incidence of twins is half of that in the United States. In the United States twins occur in Negroes with an incidence of 1.4% and in Caucasians with an incidence of 1.1%.[2] The relative frequency of multiple births increases after the age of 20, reaches a maximum in the late thirties, and then falls. Before age 20 the combined incidence of twins and triplets is 0.6%. In the age group 30 to 39 years multiple births are three times as frequent.

No exact data are available as to the frequency of conjoined twins. Mortimer and Kirshbaum[3] collected data on approximately 100,000 pregnancies from different sources and estimated that 0.6% terminated in malformed fetuses at term, and of these only a small number were conjoined twins. Scammon[4] cited 100,000 births and found 517 malformations, of which only 2 were double monsters. Potter[5] reported only one case of symmetrical conjoined twins in 60,000 deliveries at Chicago Lying-In Hospital since 1931. The comparative rarity of conjoined twins is superseded only by the occurrence of symmetrically fused fetuses in a triplet pregnancy.

A slight preponderance of boys in single pregnancies in the United States decreases with the increase in number of children per pregnancy. In triplets, boys occur with a frequency of 49.54%.

Multiple pregnancies are either monovular or polyovular. The monovular variety ranges from 25% to 33%.[2] Polyovular pregnancies result from simultaneous fertilization of two or more ova. Monovular multiple pregnancies stem from the abnormal development of a single zygote. The differentiation between a monovular and a polyovular pregnancy usually can be made if the num-

ber of amniotic sacs can be ascertained, since the number of placentas may vary from one to the number of infants delivered.

The triplets described in the present report were boys, and, since two were conjoined, a maximum of two ova were involved in this pregnancy. Whether or not the pregnancy was monovular could not be determined because of severe placental degeneration and fragmentation.

Summary

Conjoined twins occurring in an early triplet pregnancy are described. All of the triplets were boys. The twin fetuses were joined abdominally and shared only segments of the gastroenteric tract.

Sequoia Hospital District.

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Two Forms of Necrotizing Arteritis in Dogs Related to Diet and Renal Insufficiency

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Introduction

For over a decade a study has been made in our laboratory of a necrotizing arterial lesion produced in dogs by a combination of high-fat diet and renal insufficiency. Other workers have extensively described necrotizing lesions of the vascular system produced by renal insufficiency alone and not dependent on prefeeding any special diet. Frequently confusion has arisen from failure to make a distinction between the arterial lesion produced by the combination of high-fat diet and renal insufficiency and the lesion produced by renal insufficiency alone. The purpose of this report is to clarify the relationship of these two lesions.

The literature on the subject of cardiovascular disease related to the kidney, even if publications restricted to only one species were considered, is so voluminous that a comprehensive review of the literature is beyond the scope of this paper. Winternitz, in 1940,[15] in a very thorough study of lesions found in dogs subjected to various forms of renal ablation or injury, described most of the changes now commonly recognized as being associated with renal insufficiency in the dog. These included "edema, hemorrhage and necrosis of muscle, including heart muscle, smooth muscle of blood vessel walls, hollow viscera, and diaphragmatic muscle," and hemorrhagic medial necrosis in the great vessels.

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Holman, in 1941,[3] described vascular lesions occurring in dogs following uranium poisoning which characteristically involved the large elastic arteries. Subsequent investigations[4] disclosed that the essential components of the procedure necessary to reproduce this lesion consistently were (1) feeding the dogs for eight weeks or longer a diet rich in cod liver oil or creamery butter and (2) renal injury. In 1950, McAllister and Waters[6] described identical lesions produced by the feeding of viosterol to thyroidectomized dogs. Meanwhile, many other investigators[2,5,8-14] were studying the relationship of the kidneys to the blood vessels, emphasizing the necrosis of small arteries and arterioles. It was clear that such lesions could be readily produced without any particular regimen of prefeeding, although they could be aggravated by excessive intake of salt, as was shown by Orbison.[13]

Through the years, as we have attempted to study the pathogenesis and etiologic factors involved in the production of the lesions described by Holman, we have encountered in some animals necrosis of small arteries and arterioles. These lesions have occurred both with and without lesions being present in the large elastic arteries. Accurate evaluation of each animal was essential to determine the influence of alterations in the standard experimental procedure, but such evaluation was difficult when only the arteriolar lesions were present. Furthermore, we were puzzled by the facts that many other investigators studying the effects of renal insufficiency in the dog never encountered the typical lesions in large vessels that we were studying, and to the other workers

diet prior to induction of renal insufficiency seemed irrelevant to the production of arteriolar lesions.

These difficulties in reconciliation of results became more acute when our attention was focused in more recent years on the renal factor and methods of inducing renal insufficiency other than uranium nephrosis were employed in our laboratory. Some of these methods resulted in a much higher incidence than that encountered previously with uranium nephrosis. This finding directed our attention to the possibility of there being two fundamentally different types of vascular lesions related to renal insufficiency in the dog.

Materials and Methods

All of the animals used in these studies were mongrel dogs weighing from 2 to 5 kg. They were individually caged in air-conditioned animal quarters, fed once daily, and given water ad libitum. "Kennel diet" as referred to here consisted of hospital table scraps, fed in sufficient quantity to maintain growth and weight. The "high-fat diet" was composed of 24 parts beef liver, 19 parts sucrose, 19 parts corn starch, 18 parts tomato juice, 15 parts butter, 4 parts kaolin, and 1 part U. S. P. salt mixture (U. S. P. XIV, p. 789). This was essentially a low-protein high-fat diet in which 43% of the Calories were derived from fat. A weighed quantity of this diet sufficient to furnish 75 Cal. per kilogram of body weight was fed daily, and dogs so treated usually increased their body weight by about 10% over an eight-week period. The diet has been found adequate to maintain dogs in good health for periods up to two years, including periods of pregnancy and lactation.

As a result of previous studies [6] it had been found that a period of eight weeks of continuous feeding of this diet was necessary to consistently produce one of the types of arterial lesions. Occasionally dogs were fed longer than eight weeks, but the incidence of lesions was not affected. No arterial lesions of any kind other than those due to parasites or the occasional "spontaneous" arteriosclerosis of dogs have been found in dogs subjected to the diet alone.

In these particular studies a variety of methods of inducing renal insufficiency was employed. The most frequently used was the subcutaneous administration of 8 mg. of uranium nitrate per kilogram of body weight in aqueous solution (containing 8 mg. of uranium nitrate per milliliter). Occasionally this dose was ineffectual in producing azotemia and a second dose of 16 mg. per kilogram was given. Bilateral nephrectomy was performed with pentobarbital anesthesia in one step, using bilateral flank incisions and extraperitoneal resection. Bilateral ureteral ligation was performed in one step with pentobarbital anesthesia and utilization of a transperitoneal approach. Ureterocaval anastomosis was accomplished by tying a segment of polyethylene tubing into one ureter and into the iliac vein; several days later an extraperitoneal contralateral nephrectomy was done.

Vividialysis was performed on a few nephrectomized dogs at four-day intervals on a Kolf type artificial kidney.

Animals were usually killed with an overdose of pentobarbital when moribund and necropsied immediately. Those dying were necropsied as soon as they were found, usually within a few hours. Tissues from most organs were fixed in formalin, and a complete set of paraffin-embedded hematoxylin-and-eosin-stained sections was prepared. Selected blocks were stained with toluidine blue and with the Rinehart modification of the Hale stain.

Results

Qualitative Differentiation of Two Types of Lesions and Suggested Nomenclature.—All of the lesions with which we are concerned here have been quite adequately described and illustrated in previous papers from this and other laboratories. Nevertheless, since the purpose of this report is to draw a sharp line between what we believe are two fundamentally different lesions, it will be necessary to review and illustrate briefly their gross and histologic characteristics.

The first type of lesion, that which appears to be definitely related to the nature of the diet prior to the induction of renal insufficiency, characteristically occurs in the large elastic arteries and the left auricular endocardium. Figure 1 shows the earliest grossly detectable stage of this lesion in the aortic arch, and Figure 2 shows it in the left auricle. The endothelial surface is raised, finely wrinkled, and slightly opaque. Similar lesions also occur in the pulmonary artery and much less frequently may be encountered in the thoracic and abdominal aorta or in the major branches arising from the aorta. Figures 3 and 4 show an advanced stage of the lesion, with a shaggy opaque mass of

Fig. 1.—High-fat diet 12 weeks; renal insufficiency due to ureterocaval anastomosis for 6 days. This heart shows the early lesions of mucoarteritis. Note the elevated wrinkled areas on the aortic intimal surface in the ascending portion of the arch, located just above the orifice of the left coronary artery, below the orifice of the innominate artery, and on the posterior wall of the aorta.

necrotic tissue bulging into the lumen, covered in some areas by a thrombus.

Histologically the lesion begins (Fig. 5) as swelling of the interstitial spaces beneath intact endothelium and deposition of a slightly eosinophilic material which stains metachromatically with toluidine blue and strongly with the Hale stain (Fig. 6). Later, cellular and fibrillary elements undergo necrosis and many polymorphonuclear leukocytes enter the area. Calcium deposition sometimes is heavy in advanced lesions. The lesion begins in and usually involves only the inner one-third or one-half of the vessel wall or, if in the left auricle, only the endocardium. The vasa vasorum in the vicinity are not affected, and the lesion is distinctly different from the medial necrosis and hemorrhage that Winternitz [15] described in the great vessels of uremic dogs.

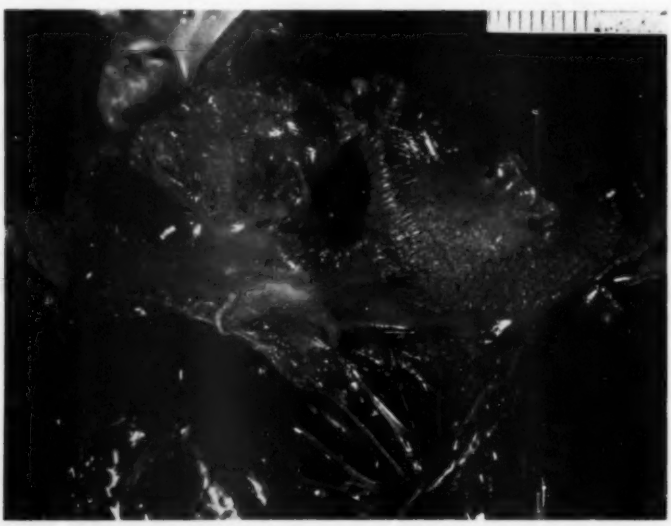

Fig. 2.—Same heart as in Figure 1, showing lesion of left auricular endocardium above posterior cusp of mitral valve similar to that in the aorta.

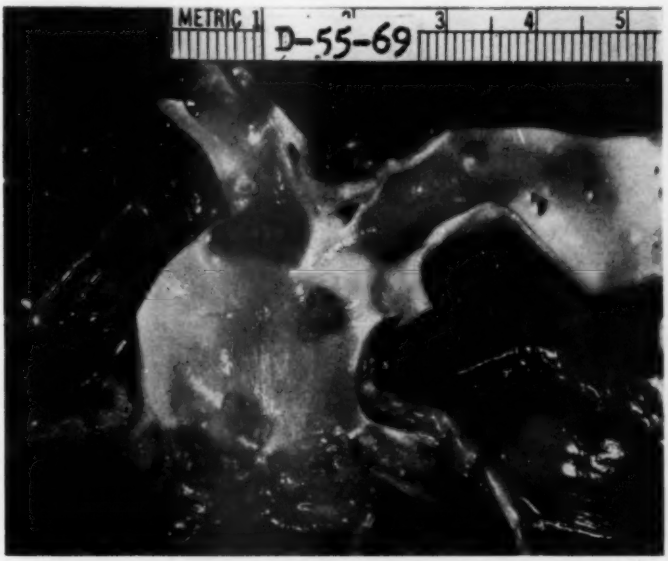

Fig. 3.—High-fat diet 13 weeks; renal insufficiency due to uranium nephrosis for 10 days. Advanced lesions of mucoarteritis in aortic arch. There are friable opaque grayish-yellow masses projecting above adjacent endothelial surface.

It has been our conclusion [7] that this lesion begins as an alteration in the connective-tissue ground substance and only later do changes appear in the fibrillary and cellular elements. For convenience and because of the primary involvement of the mucoid ground substance, we are suggesting that this form of arterial lesion be referred to as mucoarteritis.

As mentioned previously, the vascular lesions produced by renal insufficiency alone have been studied by many workers, and most of them have agreed that the most consistent lesion is necrosis of the walls of small muscular arteries and arterioles. Furthermore, most descriptions of pathogenesis have agreed that the lesion commences as necrosis of smooth muscle, as is

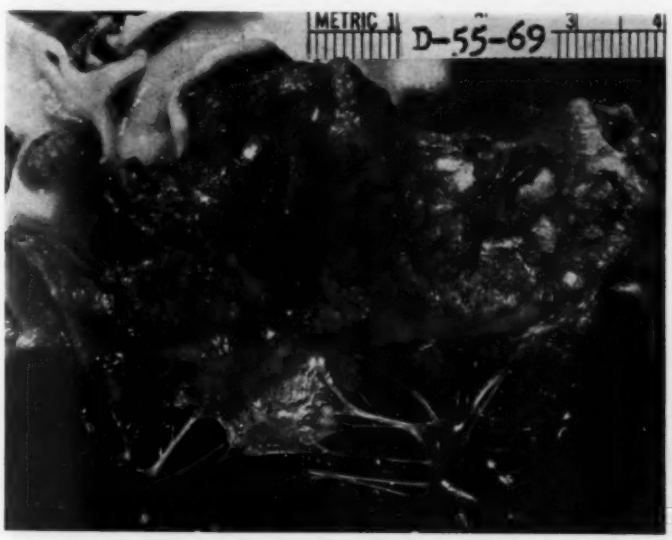

Fig. 4.—Same heart as in Figure 3, showing here extensive advanced lesions in the left endocardium.

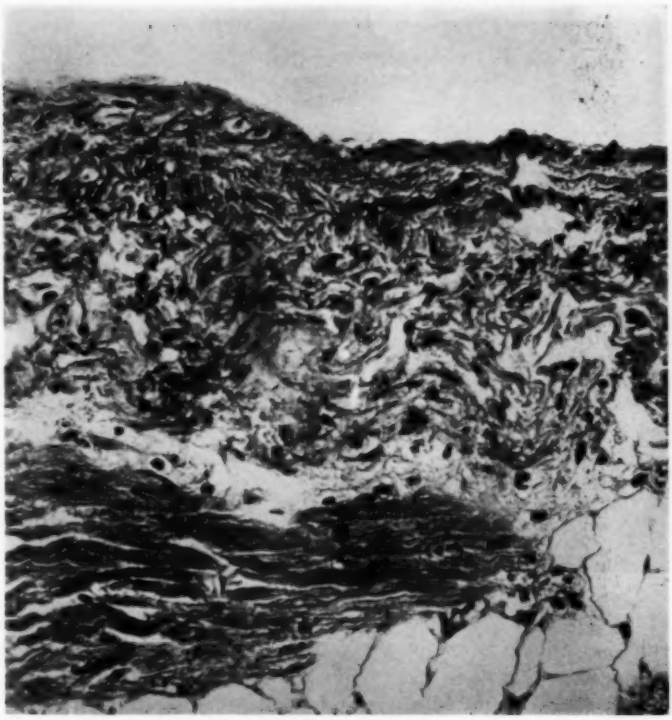

Fig. 5.—Histologic section of left auricular endocardium taken from the center of the lesion shown in Figure 2. Note the large interstitial spaces in this section and the well preserved auricular myocardium underlying it. There is little leukocytic infiltration yet. Hematoxylin and eosin (Wratten B filter [green]); reduced 47% from mag. ×600.

Fig. 6.—Consecutive section to that shown in Figure 5, stained with the Hale stain. The clear spaces seen in the hematoxylin and eosin-stained section are filled with dark-blue-staining material. Hale stain (Wratten G filter [yellow]); reduced 47% from mag. ×600.

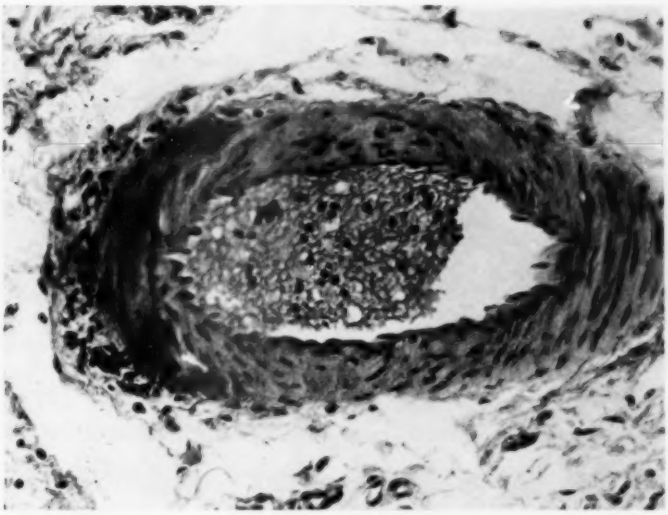

Fig. 7.—Kennel diet; renal insufficiency due to bilateral nephrectomy for seven days. Vividialysis on the fourth day. Early stage of myoarteritis. Artery in gastric submucosa, with necrosis of a portion of the smooth muscle of the outer media at the right. There is a polymorphonuclear leukocytic infiltration about the necrotic smooth muscle in the adventitia. Hematoxylin and eosin (Wratten B filter [green]); reduced 55% from mag. × 600.

illustrated in Figures 7 and 9. Even when in a mild or very early lesion there is an imbibition of interstitial eosinophilic material beneath the elastic lamella or between the muscle fibers there are also degenerative changes in the smooth-muscle cells (Fig. 8). Later, the wall becomes a ring of intensely eosinophilic coagulum (Fig. 11).

In our experience this lesion is a focal one, affecting sharply localized segments of the vessels, and the degree of leukocytic reaction is highly variable. It occurs most

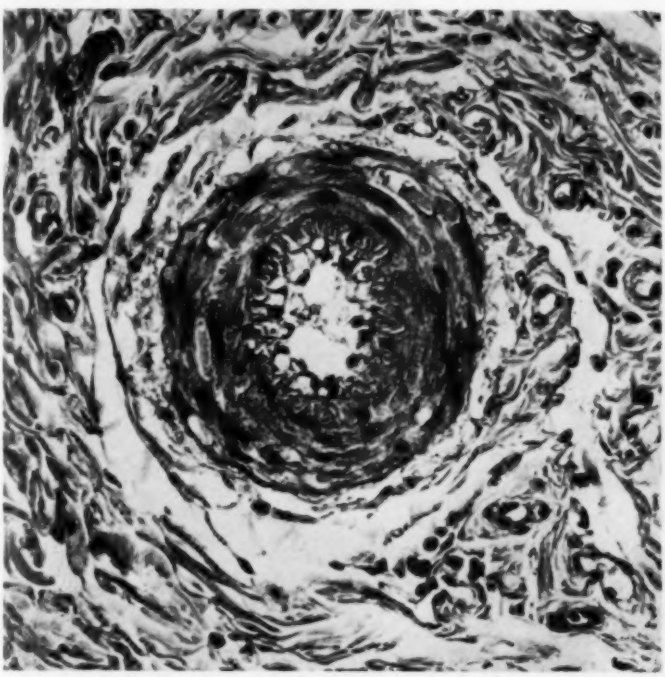

Fig. 8.—High-fat diet; renal insufficiency due to bilateral nephrectomy for six days. Early stage of myoarteritis. Artery in gastric submucosa. There is an accumulation of a granular material beneath the internal elastic lamella and between the smooth-muscle fibers, and there is also necrosis of several smooth cells. Hematoxylin and eosin (Wratten B filter [green]); reduced 35% from mag. × 600.

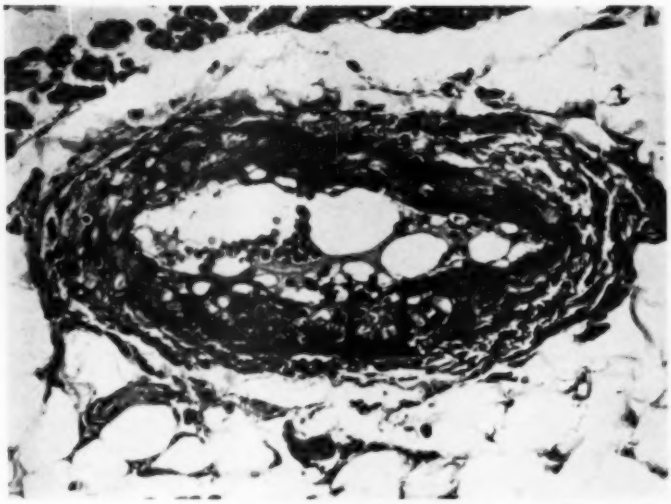

Fig. 9.—Intermediate stage of myoarteritis. This small artery lies beneath the epicardium of the left auricle, just beneath the lesion of mucoarteritis illustrated in Figure 5. There is necrosis of the smooth muscle of the entire wall, forming an intensely stained ring in place of the media. There is a slight degree of leucocytic reaction about it. Hematoxylin and eosin (Wratten B filter [green]); reduced 58% from mag. × 600.

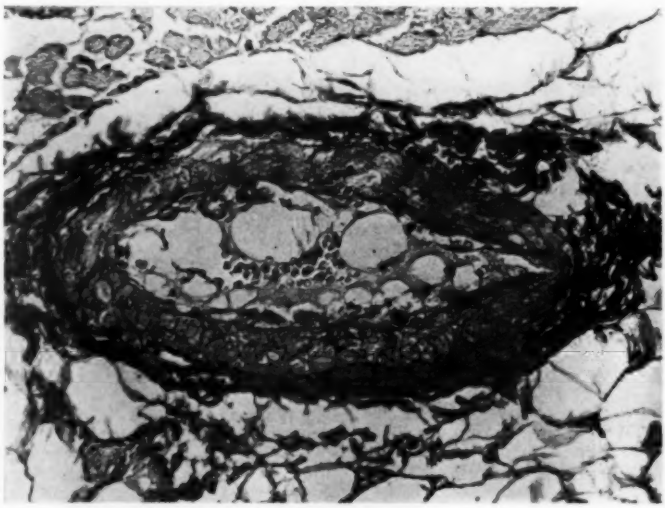

Fig. 10.—Consecutive section to that in Figure 9 demonstrating the absence of increased mucopolysaccharide in the lesions of myoarteritis. Hale stain (Wratten G filter [yellow]); reduced 58% from mag. × 600.

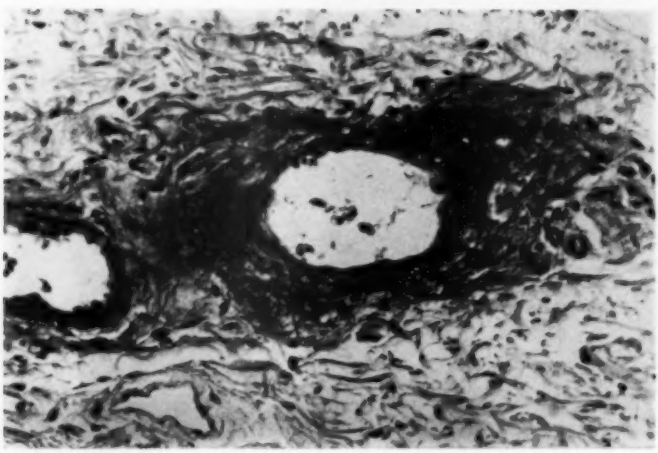

Fig. 11.—Kennel diet; renal insufficiency due to bilateral nephrectomy for 11 days; vividialysis on fourth and eighth days. Advanced lesion of myoarteritis in gastric submucosa, with extensive deposit of eosinophilic material replacing arterial wall. Hematoxylin and eosin (Wratten B filter [green]); reduced 56% from mag. × 600.

commonly in the arteries of the gastric submucosa, the colon, the gallbladder, and the urinary bladder but is also found with lesser frequency in the arteries of the small intestine, myocardium, pancreas, liver, and adrenal glands. In the acute stage the eosinophilic material replacing the arterial media is not metachromatic to toluidine blue and does not stain with the Hale stain, in contrast to mucoarteritis (compare Figs. 9 and 10).

Montgomery and Muirhead[9] have demonstrated many histochemical similarities between the material in these lesions and the sarcoplasm of smooth muscle. It seems quite possible that materials other than necrotic smooth muscle, such as plasma protein and fibrin, may escape into and be precipitated in the vicinity of the lesion, but we cannot escape the conclusion that the most prominent initial structural change is necrosis of smooth muscle. Because this type of arterial lesion appears to be so closely related to smooth muscle, and in order to contrast it with mucoarteritis, we are suggesting that it be referred to as myoarteritis.

Differentiation of Mucoarteritis and Myoarteritis by Type of Renal Injury.—Brief reference has been made to the fact that we encountered a marked difference in the incidence of myoarteritis with varying methods of inducing renal insufficiency. This difference is readily demonstrated in Table 1, in which it is seen that regardless of the diet the incidence of myoarteritis was less than 10% in all dogs subjected to uranium nephrosis (Groups 1 and 2), while in all other types of renal insufficiency (Groups 4 through 10) the incidence was consistently greater than 50%. This difference occurs despite the fact that the uranium-poisoned dogs live longer and attain levels of azotemia (as measured by the blood urea nitrogen level) comparable to those attained by bilaterally nephrectomized dogs.

All types of renal ablation or injury appear to be about equally effective in producing mucoarteritis in dogs on the high-fat diet, provided the varying times of survival are taken into account. The incidence of mucoarteritis in nephrectomized dogs is approximately 20% in dogs dying on the fourth day after nephrectomy but rises precipitously to 80% in animals which survive five days or longer. For this reason dogs dying within four days or less after nephrectomy were not included in the tabulation of Groups 3 and 4 in Table 1. We found it extremely difficult to maintain any dogs with ureteral ligation or ureterocaval anastomosis longer than three or four days, and we have excluded from the tabulation several animals which did not survive as

TABLE 1.—*Tabulation of Types of Vascular Lesions Encountered with Varying Diet and Type of Renal Injury*

Group	No. Dogs	Diet	Renal Injury	Duration of Renal Insufficiency, Days		Incidence of				Remarks
				Min.	Max.	Mucoarteritis		Myoarteritis		
						No.	%	No.	%	
1	26	High fat	Uranium nephrosis	5	16	19	73	2	8	
2	5	Kennel	Uranium nephrosis	5	15	0	0	0	0	
3	5	High fat	Bilateral nephrectomy	5	5	4	80	3	60	Only dogs surviving 5 days or longer considered
4	4	Kennel	Bilateral nephrectomy	5	5	0	0	2	50	
5	3	High fat	Bilateral nephrectomy & vividialysis	4	4	0	0	2	67	All animals died following dialysis
6	5	Kennel	Bilateral nephrectomy & vividialysis	7	11	0	0	5	100	
7	6	High fat	Ureteral ligation	3	5	0	0	4	75	Only dogs surviving 3 days or longer considered
8	4	Kennel	Ureteral ligation	3	4	1	25	2	50	
9	6	High fat	Ureterocaval anastomosis	3	6	3	50	5	83	
10	4	Kennel	Ureterocaval anastomosis	3	6	0	0	4	100	

long as three days. The brief survival time may account for the relatively lower incidence of mucoarteritis in the fat-fed dogs subjected to ureteral ligation and ureterocaval anastomosis.

Differentiation of Mucoarteritis and Myoarteritis on Basis of Diet.—It may be noted from Table 1 that out of a total of 22 dogs maintained on the kennel diet (even-numbered groups) only 1 developed mucoarteritis. On the other hand, out of a total of 46 dogs that had been fed the high-fat diet for eight weeks or longer (odd numbered groups) a total of 27 (59%) developed mucoarteritis. Even more strikingly is the relationship of mucoarteritis to the high-fat diet demonstrated if one considers only dogs with renal insufficiency for five days or longer; in such a selected group the incidence of mucoarteritis approaches 80%. Furthermore, simple prolongation of the survival time of dogs prefed the kennel diet by vividialysis (Group 6) does not precipitate mucoarteritis. In connection with this observation Muirhead[11,12] in detailed studies of a large number of nephrectomized dogs maintained for prolonged periods by peritoneal lavage did not encounter any mucoarteritis.

As for the one dog on the kennel diet with ureteral ligation which developed mucoarteritis, we do not believe that this indicates any particular effectiveness of ureteral ligation. We have found that in any large group of dogs subjected to uranium nephrosis, even though given no particular diet previously, approximately 1 out of 20, or about 5%, will develop at least minimal lesions of mucoarteritis.[4]

Comment

A summary of the distinctive gross and histologic characteristics, the relationship to diet, and the relationship to the type of renal injury of the two types of vascular lesions is outlined in Table 2. Consideration of the two lesions as fundamentally different, we believe, makes possible a satisfactory reconciliation of the discrepancies in experimental results referred to in the introduction. Other investigators have not encountered mucoarteritis because their animals were not prefed the specified high-fat diet; myoarteritis does not depend on any special diet prior to renal injury and is not produced very frequently in uranium nephrosis.

McAllister and Waters,[6] in 1950, described lesions identical to those we have defined as mucoarteritis occurring in dogs subjected to a regimen of thyroidectomy and excessive viosterol feeding. In our experience thyroidectomy has not particularly aggravated mucoarteritis. The denominators common to his procedure and ours are (1) a prolonged period of feeding a diet rich in fat (the viosterol was reported as fed in oil, 10,000 units per gram of oil, and 50,000 units of viosterol per kilogram of body weight; therefore the animals must have received about 5 gm. of oil per kilogram of body weight, or about 45 Cal. per day per kilogram, or about 60% of total required Calories from fat, as compared to 43% in Holman's diet) and (2) renal insufficiency, produced by the toxic effects of viosterol. McAllister also encountered lesions in dogs resembling those we have called myoarteritis, but, as with our animals subjected to uranium poisoning, the severity and incidence was low.

TABLE 2.—*Two Forms of Necrotizing Arteritis Related to Diet and Renal Insufficiency*

	Diet	Type of Renal Insufficiency	Location of Lesion	Histologic Pathogenesis
Myoarteritis	No special diet necessary prior to induction of renal insufficiency	High incidence with bilateral nephrectomy, ureteral ligation, and ureterocaval anastomosis; low with uranium nephrosis	Small muscular arteries and arterioles	Necrosis of smooth muscle
Mucoarteritis	Prefeeding with high-fat diet essential	Equal incidence with uranium nephrosis and bilateral nephrectomy	Large elastic arteries	Alteration in glycoproteins of ground substance

The mechanism of production of mucoarteritis has been a matter of considerable interest and continuing study in this laboratory. A possible mechanism has been suggested by the fact that certain vitamins prevent the lesions,[1] presumably supplementing or temporarily replacing a function of the proximal convoluted tubules which are destroyed by uranium. Hence any severe form of renal injury (for even ureteral ligation and ureterocaval anastomosis cause extensive structural changes in the tubular epithelium) is equally effective in precipitating mucoarteritis in fat-fed dogs.

The mechanism of production of myoarteritis is a matter of more widespread debate, particularly with respect to the importance of hypertension as a factor in the development of the lesions. The relative infrequency of myoarteritis in uranium nephrosis—even when death is apparently due to renal insufficiency—suggests that the renal factor is not related to a function of the proximal convoluted tubular epithelium but rather is related to the function of some other structure in the kidney. An investigation of the differences in the effect on renal function by uranium poisoning and, for example, ureteral ligation, might contribute to an understanding of this mechanism. Many workers in recent years have concluded that some unidentified nonexcretory function of the kidney is involved in maintenance of the integrity of the muscle of the vascular wall,[2,5,8,10] while the evidence with respect to the role of hypertension is conflicting.

It may be noted that the incidence of myoarteritis in Groups 3 through 10 is somewhat higher than that often reported in nephrectomized dogs unless the animals are given salts or renin [8,13,14] but is very close to that reported by Muirhead and associates [12] in dogs maintained for six to nine days by peritoneal irrigation. There are several factors which may account for this relatively high incidence. Since we have excluded animals dying at shorter intervals following renal injury, we have excluded a number of negative animals which others have included in their tabulations. Our animals were consistently permitted to eat as long as they would and may have ingested varying quantities of salts from either the butter or the table scraps. Furthermore, the tabulation of myoarteritis does not take into account variations in the severity of the lesions.

Attention may also be called to the difficulty encountered in prolonging the life of nephrectomized dogs which had been prefed the high-fat diet with the artificial kidney. These animals consistently died either during or a few hours after dialysis. We do not believe that this was due to faulty technique, for these animals were dialyzed after experience had been gained with the kennel-diet dogs. It seems likely that something about the prolonged high-fat diet adversely affects the response of these animals to vividialysis.

Summary

Previous reports from this laboratory have been concerned with a form of necrotizing arteritis in dogs subjected to a combination of high-fat diet and renal insufficiency, and some difficulty has arisen in attempting to reconcile the type and incidence of the lesions obtained with those described and studied in other laboratories. Reevaluation of previously accumulated material, together with comparison of the types of lesions produced following various types of renal injury or ablation, has made it possible to differentiate between two distinctly different types of vascular lesions related to renal insufficiency.

One form involves the interstitial connective tissue of large musculoelastic arteries and occurs in approximately 80% of dogs subjected to uranium nephrosis or bilateral nephrectomy if the dogs have been fed a diet rich in creamery butter for eight weeks or longer. It is suggested that this lesion be called mucoarteritis.

The other form involves primarily necrosis of the smooth muscle of muscular arteries and arterioles and is much less fre-

quent in dogs with uranium nephrosis than in those with other severe types of renal injury. It is suggested that this lesion be called myoarteritis.

Differentiation between mucoarteritis and myoarteritis related to diet and renal insufficiency in the dog makes it possible to reconcile some of the apparent discrepancies between results obtained in different laboratories.

Department of Pathology, Louisiana State University School of Medicine (12).

Miss Yvonne Stich provided technical assistance in these experiments; the Kidney Station of Charity Hospital of Louisiana in New Orleans cooperated in the use of the artificial kidney.

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The Method of Paired Comparisons in Histopathological Ranking

Hyaline Degeneration of Liver Cells

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The present paper describes the application of a new statistical technique to a histological grading problem. To set forth the purpose of the paper clearly it will be necessary to deal in the introduction with some subjects which would otherwise more easily be left to the discussion.

The question arises of the histopathological change used to illustrate the method—the appearance of hyaline degeneration in the parenchymal cells of cirrhotic liver (first described by Mallory,[1] in 1911). This was chosen because it is a relatively simple type of change, probably assessed in terms mainly of the amount of hyaline material present in the sections. It is a quality most likely, therefore, to be measurable on a linear or unidimensional scale, i. e., to be representable by a "linear variable." This condition must be satisfied if a number of objects—such as slides—are to be arranged according to the order in which they possess a particular quality, or "ranked."

Any scoring, grading, or ranking technique involves certain untested assumptions. The first is threefold—that the property evaluated is a "linear variable" (as already mentioned); that the slides differ enough for the observer to be able to distinguish between them, and that all the observers are good judges to the extent of being consistent in their comparisons. The second assumption, if the results are to be applicable by different people, is that different observers agree to some extent in their rankings or gradings. Attempts have been made in the past to test the second assumption, especially in the context of the evaluation of neoplastic processes. Something of the kind was done for the second assumption by Siegler,[2] in 1956, in comparing the diagnoses of a selected set of sections from biopsies of the uterine cervix by a number of pathologists.

The purpose of the present paper is to show how both of these assumptions have been tested in the case of a set of slides showing hyaline degeneration of liver by the application of a statistical method described by Kendall and Babington Smith,[3] in 1940—the method of paired comparisons. Those who wish to apply the method should consult the original paper by Kendall and Babington Smith.[3]

Two attributes of a set of comparisons can be evaluated by the method of these authors. The first is the internal consistence of the rankings arrived at by individual observers, in a sense, their agreement with themselves. This is measured by the coefficient of consistence, which is represented by ζ and is a measure of the legitimacy of the first (threefold) assumption referred to above. Its value lies between zero and one. If its value is at or near unity this assumption can be regarded as legitimate.

The second attribute of a set of comparisons is the agreement that obtains between different observers. The attribute of agreement or concordance is measured by the coefficient of agreement, represented by u. The coefficient of agreement is a measure of the legitimacy of the second assumption. Its maximum value is unity, and its minimum

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From the Mallory Institute of Pathology, Boston City Hospital.

TABLE 1.—*A Randomized Plan of Paired Comparisons for the Ranking of Eight Slides*

No.	Pair	Slide with More Hyalin	No.	Pair	Slide with More Hyalin
1	EG		15	DF	
2	BC		16	EH	
3	AH		17	CH	
4	DE		18	DH	
5	FG		19	FH	
6	BG		20	CE	
7	CG		21	AG	
8	EF		22	BH	
9	BF		23	AB	
10	AE		24	BE	
11	DG		25	AF	
12	AD		26	CD	
13	BD		27	GH	
14	AC		28	CF	

value is negative but varies with the number of observers. Methods for testing the significance of the values of ζ and u are available (Kendall,[4] 1955).

Materials and Methods

It was decided to compare the slides only in terms of the degree of involvement of liver cells with hyaline degeneration. Eight slides were studied by three observers. Dr. G. K. Mallory and Dr. D. Campagna-Pinto, of the Mallory Institute of Pathology, consented to evaluate the slides independently of me. The results obtained by each observer are kept separate and are identified only by a number.

All sections were of tissue fixed in Zenker's fixative with acetic acid and stained with phloxine and methylene blue, according to the method of Mallory. Sections were chosen which were thought to show a representative range of intensity of the hyaline lesion. All 28 possible pairs were then compared in a randomized sequence by each of the three observers. This was facilitated by the use of mimeographed blank forms (e. g., Table 1) in which the letter corresponding to the slide thought to show more hyalin is inserted in the blank space corresponding to each pair of slides, in the sequence indicated. It was assumed that a choice can always be made. The observer was free to study the whole slide under low and high power and to go from one slide of the pair to the other and back again several times before reaching a conclusion. A general notion of the histological definition of hyalin was assumed to be held in common, but no attempt was made to define explicit criteria of intensity in advance. The results are as set out below (Tables 2, 3, 4, and 5). Where the slide indicated by the letter in the column is thought to show more hyalin than the slide indicated by the letter in the top row, the figure "one" is inserted in the corresponding cell of the form illustrated in the Table. Otherwise "0" is inserted. The diagonal cells of the Table are left blank.

TABLE 2.—*Alcoholic Hyalin in Liver Cells—Comparisons of Observer No. 1**

	A	B	C	D	E	F	G	H	(a) Total
A	—	1	1	1	1	1	1	1	7
B	0	—	1	1	0	1	1	1	5
C	0	0	—	0	0	1	1	1	3
D	0	0	1	—	0	1	1	1	4
E	0	1	1	1	—	1	1	1	6
F	0	0	0	0	0	—	0	0	0
G	0	0	0	0	0	1	—	0	1
H	0	0	0	0	0	1	1	—	2

* If Slide A is thought to be more involved than Slide B (A→B), "1" is inserted opposite A and under B in the table. Coefficient of consistence, $\zeta = 1.0$. Ranking in descending order of involvement: A-E-B-D-C-H-G-F.

TABLE 3.—*Alcoholic Hyalin in Liver Cells—Comparisons of Observer No. 2**

	A	B	C	D	E	F	G	H	(a) Total
A	—	0	1	1	1	1	1	1	6
B	1	—	1	1	1	1	1	1	7
C	0	0	—	0	0	1	1	1	3
D	0	0	1	—	0	1	1	1	4
E	0	0	1	1	—	1	1	1	5
F	0	0	0	0	0	—	0	0	0
G	0	0	0	0	0	1	—	0	1
H	0	0	0	0	0	1	1	—	2

* If Slide A is thought to be more involved than Slide B (A→B), "1" is inserted opposite A and under B in the table. Coefficient of consistence, $\zeta = 1.0$. Ranking in descending order of involvement: B-A-E-D-C-H-G-F.

TABLE 4.—*Alcoholic Hyalin in Liver Cells—Comparisons of Observer No. 3**

	A	B	C	D	E	F	G	H	(a) Total
A	—	0	1	1	1	1	1	1	6
B	1	—	1	1	1	1	1	1	7
C	0	0	—	1	1	1	1	0	4
D	0	0	0	—	0	1	0	0	1
E	0	0	0	1	—	1	1	0	3
F	0	0	0	0	0	—	0	0	0
G	0	0	0	1	0	1	—	0	2
H	0	0	1	1	1	1	1	—	5

* If Slide A is thought to be more involved than Slide B (A→B), "1" is inserted opposite A and under B in the table. Coefficient of consistence, $\zeta = 1.0$. Ranking in descending order of involvement: B-A-H-C-E-G-D-F.

TABLE 5.—*Alcoholic Hyalin in Liver Cells—Comparisons of Three Observers Among Eight Slides**

	A	B	C	D	E	F	G	H	(a) Total
A	—	1	3	3	3	3	3	3	19
B	2	—	3	3	2	3	3	3	19
C	0	0	—	1	1	3	3	2	10
D	0	0	2	—	0	3	2	2	9
E	0	1	2	3	—	3	3	2	14
F	0	0	0	0	0	—	0	0	0
G	0	0	0	1	0	3	—	0	4
H	0	0	1	1	1	3	3	—	9

* Coefficient of agreement, $u = 0.62$. Ranking in descending order of involvement: (A, B)-E-C-(D, H)-G-F.

Results

The formulae by which the coefficients ζ and u are derived from Tables 2 to 5 are given by Kendall and Babington Smith,[3] and Kendall.[4] Tables 2, 3, and 4 summarize the results obtained by the different observers. The row totals appear in column (a) of these Tables and are used in the calculation of ζ. They are also useful as a guide to an approximate ranking of the slides. An objective ranking can be obtained by a method in which the Tables, slightly modified, are used as matrices (Kendall[5]). Such rankings are appended to the individual tables.

The coefficient ζ, the coefficient of consistence, was unity for all three observers. This means that on no occasion did any observer make an inconsistent set of comparisons, such as A> B> C> A. The values of $\zeta=1$ are highly significant at $P=0.01$, i. e., such a measure of consistency is most unlikely to occur by chance (for significance of ζ, see Kendall[4]).

The coefficient of agreement among all three observers is given by $u=+0.62$. This value can be tested for significance (for the method, see Kendall[4]). It is significant at $P=0.0^{6}32$, i. e., such a measure of agreement is exceedingly unlikely to occur by chance. The situation is similar for the coefficient of agreement among pairs of observers: Observers (1) and (2) give a coefficient of agreement of $u=+0.86$; observers (2) and (3) give $u=+0.57$, and observers (3) and (1) give $u=+0.43$. The over-all coefficient of agreement can be derived from the data in Table 5.

Comment

The above results indicate that all observers in this experiment were individually consistent. This justifies all three parts of the first assumption upon which grading and ranking must be based—that the variable is linear, the differences are perceptible, and the judges, consistent. The value of the coefficient of agreement shows that a considerable measure of agreement exists in the absence of any attempt to set up rigid criteria in advance. It is thought that the values of the coefficient of agreement among pairs of observers show that an even higher over-all coefficient of agreement could have been obtained.

The statistical method applied here, the method of paired comparisons, has been used mainly hitherto in psychological experiments and in organoleptic testing (taste testing). Its application in the present paper to a problem in histopathological ranking is believed to be the first instance of its kind. The results recorded above—obtained in the comparison of liver sections according to the degree of hyalinization of the liver cells—establish the legitimacy of the assumptions upon which the grading of "alcoholic" hyalin is based, e. g., in the paper of Phillips and Davidson.[6] This use of the method of paired comparisons introduces into pathology a new method of assessing the measure of agreement between observers, a problem raised by Siegler,[2] in 1956. It is my belief that an approach of this sort will be necessary with certain neoplastic lesions. One such situation comes to mind. It concerns the validity of histological grading of tumors. Melicow,[7] for example, in 1946, questioned the validity of the grading of cystoscopic biopsy specimens. He did not question the other assumptions involved in the use of grading procedures, namely, that the quality is measurable on a linear scale, that the differences are perceptible, that the observer is consistent, and that different observers agree. Such problems could be studied by the method of paired comparisons.

One practical difficulty is that as the number of slides studied is increased the work of making paired comparisons becomes exceedingly laborious. Alternative experimental designs suggested by Kendall,[5] in 1955, do not seem to offer much relief in this regard if the evaluation of agreement among observers is considered to be important.

Summary

The method of paired comparisons has been applied to the study of a series of

sections of cirrhotic liver with regard to their content of "alcoholic" hyalin. Three observers attained a high degree of consistence and a considerable measure of agreement. The application of the method is described in some detail. Its theoretical implications are discussed, with emphasis on the possibilities of the method in answering some questions that arise also in the morphology of neoplastic processes.

Dr. G. K. Mallory and Dr. D. Campagna-Pinto participated in the experiment and gave encouragement and advice, and Dr. S. L. Robbins read the manuscript.

Mallory Institute of Pathology, Boston City Hospital (18).

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Appendix

Calculation.—The coefficients of consistence, ζ, given above can be derived from the corresponding tables by the formulae of Kendall and Babington Smith [3] and Kendall.[4]

$$\zeta = 1 - \frac{24d}{n^3 - 4n} \text{ (where } n \text{ is even)}$$

$n = 8$ (=number of slides compared)

$$d = \frac{1}{6}\, n(n-1)(n-2) - \frac{1}{2} \sum_{i=1}^{n} a_i(a_i - 1)$$

The coefficient of agreement, u, for three observers is derived from the formulae of Kendall and Babington Smith [3] and Kendall.[4]

$$u = \frac{8\Sigma}{m(m-1)n(n-1)} - 1$$

where m=the number of observers
and n=the number of slides
$\Sigma = \Sigma^{\gamma}C_2$
γ=the number in each cell of Table 5.

Diffuse Perichondritis, Chondritis, and Iritis

Report of an Autopsied Case

T. R. HARWOOD, M.D., Chicago

In 1948, Gordon et al.[1] reported a clinical case of diffuse inflammation of cartilage, and in their review of the literature they found no previously reported similar case. These authors were unable to identify an etiological agent. We also reviewed the literature and found a number of similar cases,[2-7] although only one was autopsied.[2,3]

We have had the unique experience of 20 months' clinical observation of a similar patient followed by autopsy examination. A report of our findings was believed justified by the rarity of the condition.

Report of Case

Summary of History

A 33-year-old white city-dwelling salesman was first admitted to Vanderbilt Hospital in June, 1953, with the chief complaint of "sore ears." The patient, since December, 1952, had noticed soreness of the pinna of his left ear, without discharge, for five months. The auricle of the left ear became swollen, extremely red, and painful. In February, 1953, the patient had the same process in the right ear, with swelling, tenderness, and pain, with gradual remission. The patient had been treated with 600 r of superficial x-ray to the skin of the ear, following which his symptoms improved. In February, the patient first developed a severe cough productive of thick white material and occasionally a small amount of blood. This cough persisted throughout his illness. In March, he noted pain in the right sacroiliac region, worse in the morning but disappearing with activity. At approximately the same time the patient noticed swelling of the nose, with some redness, and about six days later noticed congestion of the conjunctivae of both eyes, without other symptoms. The patient had also noticed pain, heat, and swelling in the fourth and fifth toes of both feet. The patient had a total loss of about 80 lb. in weight prior to admission. Penicillin, streptomycin, oxytetracycline (Terramycin), and sulfonamides had been administered between January and June, 1953, for this disease, with no improvement. Fungus cultures made from the ears were reported to be negative.

Physical Findings

On admission to the hospital the patient had a temperature of 98.6 F; pulse, 120; respirations, 30, and blood pressure, 150/100. He weighed about 205 lb. The ears were swollen, and the skin, thickened, with minimal scaling and without signs of infection. Swelling almost caused occlusion of the auditory canals. There was marked hyperemia of the conjunctivae. Inconstant wheezing was heard throughout the chest, more marked on the right side. The heart was not enlarged. There was a sinus tachycardia. No murmurs were heard. The patient had mild edema of the feet and legs, with swelling of the left fifth toe and the right third, fourth, and fifth toes. An ophthalmologist in consultation found markedly congested conjunctivae bilaterally, with slight swelling in the right temporobulbar region, and haziness near the corneal limbus, with corneal vascularization. Slit-lamp examination showed keratitis punctata in the right eye, with evidence of posterior synechiae in the right eye. The fundi seemed normal centrally. The patient had a bilateral scleritis of an indolent nature, and there was an iritis of the right eye. On subsequent ophthalmological examination, no changes were found in the eye signs.

While in the hospital, the patient had a continuous sinus tachycardia. His temperature rose to 100.2 F almost every evening. He continued to have intermittent heat, redness, and swelling of the metatarsal and tarsal areas bilaterally. No etiologic diagnosis was made, and no therapy was instituted. It was decided that the patient would be followed in the outpatient clinic in an attempt to determine the etiology.

Laboratory Findings

Numerous routine urinalyses were within normal limits. The white cell count ranged from 5000 to

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18,000 cells per cubic millimeter the first few days of hospitalization, but for the remaining four weeks it was maintained at 9000 to 10,000. The differential count was within normal limits. The packed cell volume ranged from 31% to 38%, and the hemoglobin was 10.4 to 10.8 gm. per 100 cc. The corrected sedimentation rate was 29 to 30 mm. on repeated determinations. The platelet count was normal. A phenolsulfonphthalein excretion test showed a total of 90% excretion in two hours. Two tests were negative for porphyrins in the urine. Three L. E. preparations were negative for L. E. cells. A filter-paper electrophoretic pattern on the serum was considered normal. The plasma nonprotein nitrogen; fasting blood sugar; carbon dioxide, and serum chloride, sodium, potassium, calcium, phosphorus, alkaline phosphatase, and bilirubin were all within normal limits. There was a hyperglobulinemia, with an albumin-globulin ratio of about 1/1, but the total serum proteins were within normal limits. A cephalin flocculation was negative, and thymol turbidity was normal. The uric acid and cholesterol were within normal limits. Kahn, Wassermann, and VDRL tests were all negative. The histoplasmin complement-fixation test was negative. The old tuberculin skin test was positive at a 1:200,000 dilution, and a histoplasmin skin test was positive at a 1:100 dilution. The vital capacity was 96% of normal. No acid-fast bacilli were seen in three sputum smears, and none grew on culture. Four blood cultures contained Staphylococcus pyogenes var. albus ranging from 1 to 7 colonies per cubic centimeter. These were interpreted as contaminants and five subsequent blood cultures were negative. Nasal scrapings for lepra bacilli were negative. An electrocardiogram showed only a sinus tachycardia.

Soft-tissue films of the ears and nose showed loss of the usual ridges produced by cartilaginous structures. Intravenous pyelograms were normal. Two chest x-rays were within normal limits. The knees and shoulders showed no bone or joint abnormalities. The skull x-ray was normal. An x-ray of the spine showed only some flattening of the normal lordotic curve. The pelvis showed mild to moderate diffuse decalcification of the bones of the pelvis. X-rays of the hands were negative for significant bone or joint disease at this time. X-ray of the right foot showed all the bones to be moderately decalcified, with the distal portions showing severer decalcification. The heads of the metatarsals manifested a great deal of subarticular erosion, with the involved areas assuming a punched-out appearance in several of these bones, particularly the third metatarsal. There was a similar destructive change involving the base of all the proximal phalanges, with the change least marked in the proximal phalanx of the great toe. There was extensive periosteal thickening involving all the proximal phalanges except that of the great toe. X-ray of the left foot showed similar changes in the fifth metatarsal and in the proximal phalanx of the fifth toe.

A biopsy specimen from the right ear showed hyalinized connective tissue in the corium, with some plasma cells and lymphocytes, and cartilage with markedly degenerative changes. The cartilage had lost its homogeneous staining characteristics and focally was more basophilic than normal. Stains for Mycobacterium leprae revealed no bacilli. The final diagnosis on this biopsy specimen was as follows: degenerative changes of cartilage, cause unknown. A second biopsy specimen, from the right costochondral junction, showed a slight increase of eosinophils in the marrow and cortex. A portion of the tissue microscopically resembling tendon (possibly intercostal muscle attachment) contained chronic inflammatory cells. No cartilage was identified in the sections.

Follow-Up

The inflammatory reaction in the patient's eyes began to improve after two months. The metacarpophalangeal joint of the right index finger became painful and swollen. X-ray of the hand again revealed no definite bony changes. The patient's appetite improved, and he gained about 10 lb. during this period.

During the one-month interval between his last visit to the outpatient clinic and the time of death, the joint symptoms had apparently improved. However, two attacks of sudden shortness of breath were reported. On the day of his death the patient developed a sudden onset of dyspnea with cyanosis and died before the family could bring him to the hospital.

No final clinical diagnosis was made on this patient other than "diffuse cartilagenous disorder with symptoms of 20 months' duration." The terminal event suggested obstruction of the laryngeal or tracheal airway.

Summary of Autopsy Findings

Gross Examination

The body was that of a well-developed very obese white man, appearing the recorded age of 33 years, weighing about 230 lb., and measuring 5 ft. 11 in. in length. Autopsy was begun four and one-half hours after death.

Fig. 1.—Front and side views of patient, showing loss of usual contours of nose and ears due to destruction of cartilage.

The skin was smooth and showed no lesions. Both ears had a "cauliflower" appearance. Both auditory canals were almost occluded. The nose was flattened, and the face had a somewhat leonine appearance (Fig. 1). No cartilage was felt in the nose, although both nares were patent. The trachea was midline but lacked normal rigidity. The remainder of the external examination revealed no gross abnormalities.

Internal examination revealed no polyserositis. The lymph nodes were generally enlarged. The tracheal lumen was almost completely occluded (Fig. 2), owing to thickening of the wall and what appeared to be collapse from complete destruction of tracheal cartilage. The destruction of cartilage was generalized throughout the ears, the shoulders, the hips, the vertebra, and the joints of the legs, with no normal cartilage being found grossly. There was an old calcified Ghon lesion in the right lung. Hilar lymph nodes showed small areas of caseous necrosis, but no other evidence of active tuberculosis was found. There were an acute suppurative bronchopneumonia, acute splenitis, and swelling of the liver and kidneys compatible with passive congestion.

Permission to examine the brain was not granted.

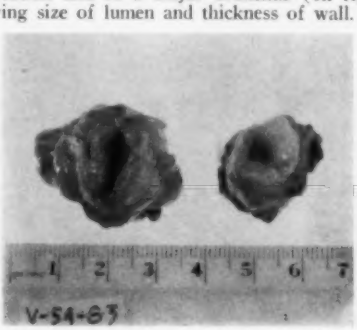
Bacteriologic Findings

Blood-agar plates inoculated with material from lymph nodes remained sterile. Blood cultures obtained at autopsy showed 10 to 12 colonies per plate of S. pyogenes var. albus and 10 to 12 colonies of nonpathogenic diphtheroids. Guinea pigs inoculated with material from the caseous hilar nodes resulted in demonstration in one animal only of a few acid-fast bacilli exhibiting the morphology of tubercle bacilli. None of the organisms could be found in sections exhibiting lesions of the cartilage, and cultures of the lesions made at autopsy were sterile.

Microscopic Examination

Microscopic sections confirmed the gross findings in the lungs, liver, and kidneys. The spleen and lymph nodes showed a nonspecific hyperplasia. The lesions of cartilage, similar in various parts of the body, will be the only changes described (Figs.

Fig. 2.—Cross section of trachea (on left) at bifurcation and of a major bronchus (on right), showing size of lumen and thickness of wall.



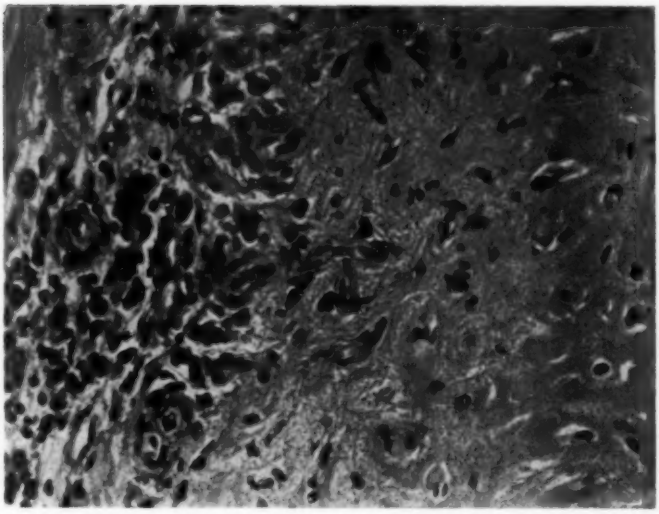


Fig. 3.—Photomicrograph of ear cartilage, showing inflammatory reaction, with infiltration of plasma cells and lymphocytes. The irregularity of staining in the cartilage matrix is evident. Hematoxylin and eosin; reduced 15% from mag. × 374.

3, 4, 5, and 6). The cartilaginous areas were largely replaced by fibrous tissue (some of which was calcified), with infiltration of plasma cells and lymphocytes. The remnants of cartilage showed a lack of homogeneity of the ground substance and a variety of chondrocytes, some with basophilic cytoplasm and hyperchromatic nuclei. The ground substance in hematoxylin-and-eosin sections had a pinkish-blue color and in some areas was fragmented and contained nests of cartilage cells surrounded by a thickened faintly eosinophilic fibrous tissue. The periodic acid-Schiff stains of cartilage-containing areas were only faintly and irregularly pink, confirming the presence of defective ground substance in the cartilage with replacement by dense collagenous tissue. Silver stain for spirochetes showed none in the lesions.

Death was ascribed to the bronchopneumonia due to the loss of adequate airway and drainage resulting from gradual tracheal collapse and thickening.

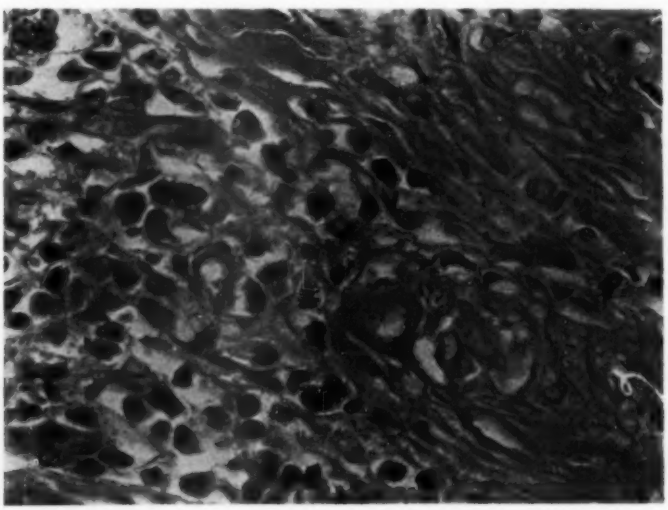


Fig. 4.—Photomicrograph of tracheal cartilage with inflammatory lesion similar to the reaction seen in the ear (Fig. 3). Hematoxylin and eosin; reduced 15% from mag. × 624.

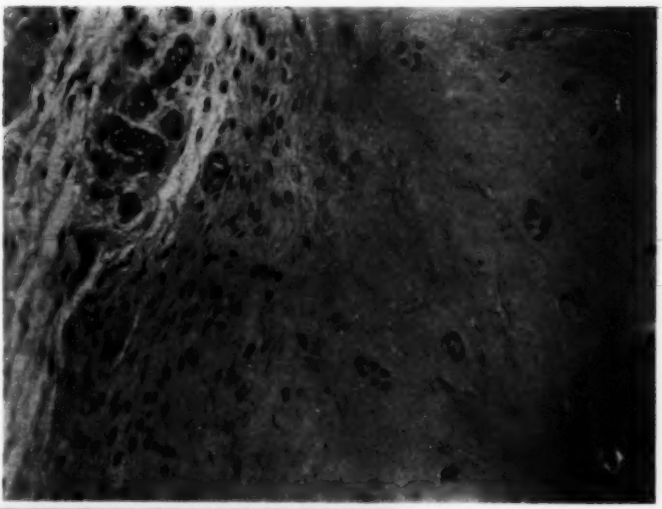


Fig. 5.—Photomicrograph of sternal cartilage exhibiting nests of cartilage cells with as many as six cells per nest and showing lack of homogeneity of ground substance as well as nonspecific inflammation. Hematoxylin and eosin; reduced 15% from mag. × 187.

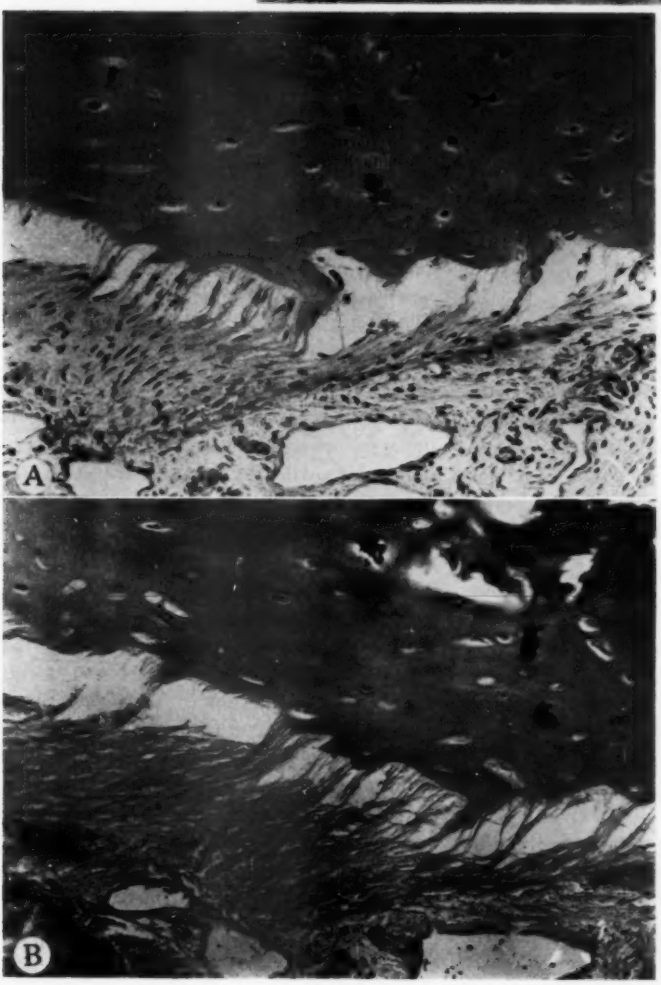


Fig. 6.—Photomicrographs of intervertebral disk cartilage. Both show nonspecific fibrous-tissue reaction adjacent to cartilage exhibiting irregularity of staining of ground substance; reduced 15% from mag. × 187. *A*, hematoxylin and eosin. *B*, periodic acid-Schiff reagent.

Comment

A review of the literature revealed a number of reports [8-13] in which local perichondritis or local chondritis were found in human patients; but the cases of similar generalized disease reported by all others [2-7] and the present case total only six. The only other autopsied case, reported by Altherr,[2,3] died of miliary tuberculosis. In none of the cases has the etiology of the cartilaginous change been determined.

In the presently reported case it has been impossible to determine an etiologic agent or define the pathogenesis satisfactorily. The tubercle bacillus was suspected, but except in a hilar lymph node it could not be demonstrated in any part of the body. Gordon et al.[1] suspected that the Treponema pallidum or gonococcus might be responsible in their case, although adequate evidence was lacking. In the presently reported case, not only was there no history suggestive of syphilitic infection but no serological or morphological evidences of syphilis were found and no spirochetes were demonstrable in the lesions. There was nothing to suggest a gonococcal infection.

The possibility of a congenital abnormality is unlikely because of the marked chronic inflammatory reaction and destructive character of the lesions. Bacterial agents (other than possibly the mycobacteria) may be ruled out, since none could be cultured from the lesions or demonstrated in the tissue sections. The S. pyogenes var. albus bacteremia was certainly a secondary phenomenon. No other pathogen was implicated.

Other authors [4,7,14] have suggested hypersensitivity as an explanation of pathogenesis. Is it possible that a hypersensitivity to one's own cartilage could lead to so destructive a process and such a marked inflammatory reaction? Bacsich and Wyburn [15] have failed to produce hypersensitivity in experimental animals to either cartilage or cornea. Our attempts to produce such a reaction in guinea pigs, using their own cartilage, have also failed. Cartilage was removed from six male guinea pigs. Some of the cartilage was killed by heating and immediately replaced in the subcutaneous tissues of the back. The remainder of the cartilage was pooled and ground in a mortar; 250,000 units of penicillin and 0.05 gm. of streptomycin were added to half of the material, and the suspensions were stored in the Deepfreeze until used. No reactions were obtained with subcutaneous injections of the cartilage suspension (and antibiotics), either alone or in conjunction with Freund's adjuvant. A suspension of the cartilage without antibiotics likewise failed to produce reactions. In all cases the sensitizing injections contained no antibiotics, while some of the challenging injections contained antibiotics. Tissues obtained from the killed animals after four months showed no lesions similar to those in the case reported.

In spite of our experimental results, a hypersensitivity reaction seems the best hypothesis. It is possible that an abnormal cartilage in the human cases (as shown by the periodic acid-Schiff-staining reaction) acted as an antigen to induce a hypersensitivity, although "normal" cartilage in experimental animals did not.

There is one other hypothesis which will fit the known facts. It is possible that the hypersensitivity was produced in the cartilage and cornea by bacterial infection in a similar manner to that of so called "collagen diseases," and the fact that the organisms responsible could not be cultured over a 20-month period does not invalidate the hypothesis. Why certain organs or organ systems are affected in the "collagen diseases" is still not understood, but it is conceivable that in some hosts the body cartilage and the cornea might be the tissues affected. Since the cartilage and cornea are embryologically similar, they might be affected as a unit in a manner similar to the connective tissues of the vascular system in polyarteritis. In two cases reported by others [6,7] there was no apparent tuberculin hypersensitivity while streptokinase or

streptolysin titers were high. This would lend support to the hypothesis of a rheumatic pathogenesis in this syndrome. The work of Glynn and Holborow,[16] showing the production of hypersensitivity to chondroitin sulfate following intravenous inoculations of chondroitin sulfate-streptococcal antigen, would further suggest that syndromes like that recorded in this paper are similar to the "rheumatic or collagen diseases." Their observation is consistent with the possibility that this case represents a hypersensitivity of the patient to his own tissues initiated by infection.

Summary and Conclusions

An autopsied case of diffuse chondromalacia, generalized chondritis, and perichondritis with associated eye disease is reported.

A definite etiological agent could not be identified.

The pathogenesis is obscure. It is suggested that the syndrome is a manifestation of hypersensitivity.

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The Influence of Tissue Extracts on the Growth of Ehrlich Tumor

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Recently we observed that during the early stages of development subcutaneously implanted Ehrlich tumor grew at strikingly different rates in CAF_1 hybrid and Strong A mice. In contrast, no marked difference in growth rate was observed for the two host strains when the tumor was implanted in the muscle or in the peritoneal cavity. These data suggested that the temporary delay of tumor growth in the subcutis of Strong A mice might be due to some local factor or factors inhibiting the mitotic activity of the rapidly propagating neoplastic cells. Our experimental data on the growth of the tumor in different tissues of the hosts suggested that the inhibitory effects of systemically active substances like antibodies or properdin are not likely to be responsible for the observed difference.[1]

The present investigation seeks to establish the presence of a mitotic inhibitor in Strong A mice which is capable of lowering the protoplasmic viscosity of Ehrlich ascites tumor cells. Furthermore, it correlates the phenomenon of mitotic arrest with the reduced viscosity state in these cells. Evidence recently presented suggests that substances like colchicine and Serratia marcescens polysaccharide, which induce a lowering of the protoplasmic viscosity in tumor cells, interfere with the formation and function of the achromatic spindle during mitosis.[2]

Heilbrunn, Wilson, and Harding[3] demonstrated that heparin-like extracts obtained from tissues of certain marine organisms are mitotic inhibitors of dividing sea-annelid and sea-urchin eggs. We therefore considered it worth while to attempt the extraction of polysaccharides from the subcutaneously grown tumors and the surrounding tissues of Strong A mice and to compare their biological activities with material identically prepared from the CAF_1 hybrid strain.

Materials and Methods

As in our previous investigations, Ehrlich ascites tumor propagated from a line obtained in 1953 from Dr. Robert Schrek, of Hines Veterans' Administration Hospital, was used. The stock tumor was maintained by weekly intraperitoneal transfers of 0.2 ml. aliquots of the ascitic suspension into Strong A mice of both sexes, 3 to 6 months old. All mice used in this study were given unrestricted intake of Rockland Mouse Pellets and water in air-conditioned quarters.

I. Tumor Growth in CAF_1 Hybrid and Strong A Mice.—The mice used to determine the rate of growth of Ehrlich tumor in the various body sites were CAF_1 hybrid and Strong A strains 3 to 6 months of age. The CAF_1 hybrid mice were obtained from Dr. Willard T. Hill, Department of Pathology, and Strong A mice were maintained for several generations from stock animals originally supplied by Dr. Guy P. Youmans, Department of Bacteriology, both of Northwestern University Medical School.

A. Tumor Growth in Subcutaneous Tissue: A tumor suspension obtained from male Strong A donor mice bearing a 10 days' ascitic growth was diluted with sterile isotonic saline solution so that the final preparation contained 10,000,000 viable tumor cells per 0.2 ml. A total of 45 CAF_1 hybrid (14 males and 31 females) and 42 Strong A (13 males and 29 females) mice were injected aseptically with 10,000,000 tumor cells (0.2 ml.)

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TABLE 1.—*Comparison of Wet Weights of Subcutaneous and Intramuscular Ehrlich Tumor in CAF_1 and Strong A Mice*

	Subcutaneous		Intramuscular	
	CAF_1	Strong A	CAF_1	Strong A
No. of mice	45	42	31	32
Mean tumor wet wt., gm.	2.8	0.9	3.3	2.1
Standard error	±0.2	±0.1	±0.1	±0.1
Ratio: CAF_1/Strong A	3.0		1.6	

subcutaneously in the interscapular region. The mice were divided equally into three groups for each strain, and all mice were killed with an excess of ether according to the following schedule: Group I after 9 days' growth, Group II after 10 days' growth, and Group III after 11 days' growth. The tumor from each mouse was dissected free of the surrounding tissues, and the wet tumor weights were tabulated (Table 1). After the tumors were weighed, the skin surrounding the neoplasm was scraped to remove the subcutaneous fibroadipose tissue. The tumor and the subcutaneous scrapings for the two host strains were pooled separately and immediately frozen for the extraction of polysaccharides (II. A., below).

B. Comparison of Intramuscular Growth of Ehrlich Tumor: Thirty-one CAF_1 and thirty-two Strong A mice, all males, were given intramuscular injections of 0.2 ml. (10,000,000 viable cells) into the left thigh. Twelve mice from each strain were killed after 9 days; nineteen CAF_1 hybrid and twenty Strong A mice were killed after 11 days. Both hindlimbs from each mouse were disarticulated at the acetabular joint, and the skin was removed down to the feet. Wet weights of the left hindlimb (tumor-bearing) and the right hindlimb (normal) were recorded separately for each animal. The difference in weight between the two hindlimbs was considered the wet tumor weight for a given animal. The results are tabulated in Table 1.

C. Comparison of Rate of Intraperitoneal Growth of Ehrlich Tumor in CAF_1 Hybrid and Strong A Mice: Fifty male CAF_1 hybrid mice were given 2,000,000 viable tumor cells (in 0.2 ml.) intraperitoneally under aseptic conditions. The samples of ascitic-fluid suspension from five mice killed on each of the days indicated in Figure 1 were examined for the estimation of mean total tumor cells per mouse, as previously described.[4] The cube-root growth curve for male CAF_1 hybrid strain was compared with the curve obtained earlier for Strong A mice (Fig. 1).

II. Extraction of Tissue Polysaccharides.—A. Extracts from Tumor and Surrounding Subcutaneous Tissues: For a pilot study the frozen tumor and subcutaneous tissue of four mice from Strong A strain were thawed and ground with 1.5 ml. of distilled water per gram of wet tissue in the

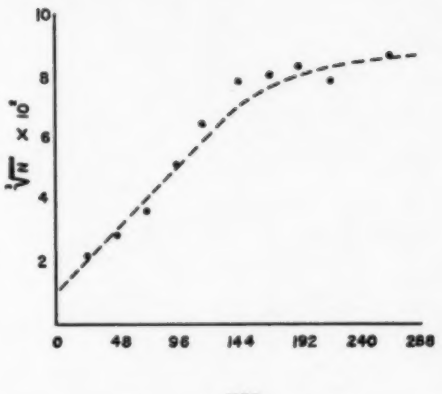

Fig. 1.—Each solid circle represents the mean N value for five CAF_1 hybrid mice. Inoculum of 2,000,000 viable Ehrlich ascites tumor cells was given intraperitoneally ,to each animal. Broken line expresses the mean growth curve of the tumor in Strong A mice. N indicates total tumor cells.

Waring Blendor for 10 minutes. The preparation was filtered through Whatman No. 40 filter paper, and the filtrate (TP1A) was used to determine the effect on the protoplasmic viscosity of Ehrlich ascites tumor cells by in vitro tests. The protoplasmic viscosity test was carried out by mixing 0.2 ml. of the crude aqueous extract with 0.5 ml. of the ascitic tumor suspension from male Strong A mice bearing the ascitic tumor, with use of the centrifugation technique.[9,8]

The subsequent extraction procedures were carried out in identically the same manner for CAF_1 hybrid and Strong A tissues. About one-half of the remaining Strong A frozen tumor and subcutaneous tissues were thawed, weighed, and ground in the Waring Blendor for 10 minutes, with the use of 1.5 ml. of distilled water per gram of wet tissue weight. The brei was strained through a gauze, and the solution was treated with 1/5 vol. chloroform and 1/15 vol. isoamyl alcohol to defat and precipitate the proteins from the preparation, as described by Sevag.[6] Then the solution was filtered through Whatman No. 40 filter paper and the filtrate was transferred into a separatory funnel. An equal volume of chloroform was added to the filtrate and the contents of the vessel agitated. The chloroform layer containing the extracted fat and the excess of isoamyl alcohol was separated from the aqueous fraction, and the chloroform extraction was repeated at least three times. The pH of the water-soluble fraction was adjusted finally to 7.2, with 0.1 N NaOH, then lyophilized for storage. This lot was designated TP2A for the fraction from Strong A mice. A small amount of the powdery lyophilized material from TP2A was dissolved in distilled water so as

to contain 4.0 mg. per milliliter of the crude substance.

The remainder of the frozen tissues from Strong A and CAF_1 hybrid mice were processed as described above, but the solutions containing 4.0 mg. per milliliter of the lyophilized material was dialyzed for 24 hours against distilled water at 3 C. These preparations were designated TP3A and TP3B for Strong A and CAF_1 tissues, respectively, and were frozen until ready to use.

TP3A and TP3B were examined for evidences of sugars and reducing groups, with use of the following methods: 5-hydroxymethylfurfural test,[7] phenolsulfuric acid reaction,[8] and periodic acid-Schiff reaction.[9] Resorcinol (Seliwanoff test)[10] and naphthoresorcinol[11] tests were performed on the preparations after hydrolysis with concentrated sulfuric acid.

B. Extracts from Subcutaneous Tissues After Silica Gel Injection: To determine whether the biologically active polysaccharide-like fraction resides in the tissues of Strong A mice which do not bear the transplanted subcutaneous tumor, a foreign-body reaction was induced under the skin of these mice with silica gel and the chemical extraction procedure was carried out on the subcutaneous tissue. Silica gel * was washed with distilled water and resuspended in 0.85% NaCl to make a preparation containing 0.3 gm. per milliliter. Five female Strong A mice were given subcutaneous injections of 0.5 ml. of the suspension in the interscapular region. The animals were killed after nine days. The area surrounding the foreign-body reaction was dissected free and the subcutaneous fibroadipose tissue was scraped from each specimen. The tissue samples were pooled for the extraction of the polysaccharides by Sevag's method, and the water-soluble fraction was treated in the manner described in Section II. A., but the pH was not adjusted to 7.2 and the material was not stored in a frozen state. This preparation was designated TP4A.

III. Histologic Studies.—Ten CAF_1 hybrid and ten Strong A mice, all males, were given subcutaneous inoculations of 10,000,000 viable tumor cells, as in section I. A. One mouse from each strain was killed daily for 10 days. Three blocks were taken from each animal, to include the skin, the underlying tumor, and the surrounding subcutaneous tissue. The first of the three blocks was fixed in Bouin's solution; the second, in alcohol-formalin fixative, and the third was fixed by freezing in isopentane chilled with liquid nitrogen. The present studies include only those sections prepared from the first and second blocks. After they were embedded in paraffin, sections from the blocks were cut 5μ to 8μ thick. The prepared sections were stained with hematoxylin and eosin, Mallory's aniline blue, toluidine blue, Best's carmine, methyl green-pyronin Y, and alcian blue-periodic acid-Schiff stains.

Three CAF_1 hybrid and three Strong A mice, all males, were inoculated subcutaneously with 0.5 ml. of the silica-gel suspension described in Section II. B. One mouse from each strain was killed after four days, and the remainder, after nine days. All specimens were removed, fixed, sectioned, and stained as described in the foregoing paragraph.

IV. Determination of Protoplasmic Viscosity Values and Mitotic Indexes of Ehrlich Ascites Tumor Cells in Strong A Mice After Injection of Tissue Extracts.—Strong A mice of both sexes were inoculated with 0.2 ml. of ascitic tumor suspension from a donor mouse. The tumor was permitted to develop from 7 to 14 days. Tumor growths of less than seven days were avoided because the base values of the mitotic index were found to be too variable from one animal to the next. Furthermore, the ascitic volume in mice with young tumors was generally inadequate for repeated sampling. The mean age of the tumor used for this series was nine days.

Determinations of protoplasmic viscosity and mitotic index were made in the manner described in an earlier publication.[2] Briefly, the ascitic-fluid sample from each tumor-bearing mouse was aspirated, and the initial relative viscosity value (VV) † of tumor cells was determined. The base value for the mitotic index (MI) was estimated from the same sample. Then the mice were given a single injection of the test substance, and subsequent ascitic-fluid samples of 0.1 to 0.2 ml. were analyzed sequentially for the relative protoplasmic-viscosity value and the mitotic index at appropriate intervals. For the determination of mitotic index the technique used in the present study was simplified. The cells were examined for mitotic figures on fresh preparations in which a small drop of ascitic-fluid suspension was placed on a microscopic slide and covered with a coverglass in the usual manner. After the cells were flattened the preparation was examined under the dark-contrast oil-immersion lens of the phase-contrast microscope. The mitotic figures were identified readily and were defined clearly. In many respects this method was found to be superior to the smearing and staining technique previously described by us.[2]

A. TP2A: For a pilot experiment two female Strong A mice with Ehrlich ascites tumor were given a single intraperitoneal injection of 0.2 ml. of TP2A after thawing. Samples were aspirated from the peritoneal cavity for the determination of VV and MI prior to the injections of TP2A and at intervals shown in Tables 2 and 3.

* The Davison Chemical Corporation, Baltimore 3, Md.

† See Reference 2 for definition.

TABLE 2.—*Effect of Tissue Extract from Strong A Mice upon Viscosity Values of Ehrlich Ascites Tumor Cells in Strong A Mice*

Animal No.	Hours							
	0	¼	5	6–7	9	12	24	26–30
TP2A-1	0.96	0.56	0.65				0.76	
TP2A-3	0.94	0.60	0.80			0.97		
TP3A-1	0.99	0.67	0.71	0.65	0.73	0.95	0.93	
TP3A-2	0.98	0.46	0.55	0.61	0.58	0.77	0.99	
TP3A-4	1.00	0.63	0.71	0.71	0.79	1.06	0.96	
TP3A-6	0.93	0.64	0.67	0.78	0.78	1.02	0.94	
TP3A-7	1.00	0.66	0.73		1.06		1.03	
TP3A-8	1.00	0.65	0.77		0.91	0.94		0.95
TP3A-9	0.95	0.50	0.53		0.71	0.92	0.93	
TP3A-10	0.95	0.62	0.71		0.92		1.00	
TP3A-11	0.94	0.56	0.62		1.01			
TP3A-12	0.97	0.75	0.71		0.94			0.94
TP3A-13	0.97	0.66	0.81		0.95			0.97
TP3A-14	1.00	0.69	0.79		1.01		1.02	
TP3A-15	1.05	0.66	0.63		1.03		0.95	
TP4A-1	0.87	0.56	0.62		0.54	1.00	1.02	
TP4A-2	0.99	0.65	0.68		0.73	1.01	1.00	
Mean VV	0.97	0.62	0.69	0.69	0.85	0.96	0.96	0.95
Std. Error	0.01	0.02	0.02		0.04	0.03	0.02	

B. TP3A: Seventeen Strong A mice of either sex bearing Ehrlich ascites tumor were given one injection of 0.2 ml. of TP3A intraperitoneally. The ascitic-fluid samples were taken just before the injections and at intervals shown in Tables 2 and 3 for the estimation of VV and MI.

C. TP3B: Fourteen Strong A mice of either sex bearing the ascitic tumor were given a single injection of 0.2 ml. of TP3B intraperitoneally.

TABLE 3.—*Effect of Tissue Extract from Strong A Mice upon Mitotic Index of Ehrlich Ascites Tumor Cells in Strong A Mice*

Animal No.	Hours								
	0	5	6	7	8	9	12	24	26–30
TP2A-1	1.2	0.9						4.4	
TP2A-3	1.8						3.5		
TP3A-1	1.2	1.3		3.8		6.1	4.4	2.6	
TP3A-2	1.2	1.0		4.6		5.1	6.0		
TP3A-4	1.2	1.6	4.0			5.0	4.1	1.0	
TP3A-6	0.9	1.3	2.3			4.3	2.6	4.1	
TP3A-7	2.0	4.5				3.6		2.4	
TP3A-8	1.8	1.0	0.8			1.4	1.9	4.9	1.9
TP3A-9	2.7	3.2	3.8			5.0	4.8	4.1	
TP3A-10	1.8	2.5		0.6		0.7		3.7	
TP3A-11	1.5	2.7		5.9		3.0			
TP3A-12	1.0	2.5		6.5	4.2	4.1			3.0
TP3A-13	2.0	3.1		5.5	4.5	4.7			4.9
TP3A-14	2.7	3.0	2.7	4.5	4.1	2.2		2.8	
TP3A-15	1.3	3.1	3.7	4.4	4.2	2.5		3.3	
TP4A-1	1.5	2.7	2.8			4.3	3.0	3.6	
TP4A-2	1.0	1.6	2.0			4.4	1.0	1.1	
Mean MI	1.5	2.2	2.8	4.5	4.2	3.8	3.5	3.2	3.3
Std. Error	0.2	0.3	0.4	0.7		0.4	0.5	0.4	

The ascitic-fluid samples were aspirated prior to injections and at intervals indicated in Tables 4 and 5 for the determination of VV‡ and MI.

D. TP4A: Two male Strong A mice with the ascitic tumor (TP4A-1 and TP4A-2) were given a single intraperitoneal injection of 0.2 ml. of TP4A. Samples for VV and MI determinations were taken prior to injections of TP4A and at intervals shown in Tables 2 and 3. Subsequently TP4A was inadvertently kept unfrozen and was

‡ The determination of VV was omitted in animals TP3B-12, TP3B-13, and TP3B-14.

TABLE 4.—*Effect of Tissue Extract from CAF_1 Mice upon Viscosity Values of Ehrlich Ascites Tumor Cells in Strong A Mice*

Animal No.	Hours					
	0	¼	5	9	12	24
TP3B-1	0.93	0.92	1.00	0.99	0.99	1.01
TP3B-2	1.00	0.97	1.03	1.00		
TP3B-3	1.00	0.98	1.04	0.96	0.98	0.96
TP3B-4	0.93	0.95	1.00	0.97	0.96	
TP3B-5	0.98	1.03	0.99		1.01	0.95
TP3B-6	1.03	1.00	0.97			0.97
TP3B-7	0.97	1.01	0.96		1.00	1.00
TP3B-8	0.91	0.97	1.00	0.94	1.05	0.94
TP3B-9	0.97	0.95	1.05	0.98		0.96
TP3B-10	0.96	1.00	0.95	0.97		0.96
TP3B-11	0.98	1.00	0.97	0.94		0.94
Mean VV	0.97	0.98	1.00	0.97	1.00	0.97
Std. Error	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

stored in the refrigerator at about 5 C for one month. After storage this preparation was found to be inactive when in vivo tests for protoplasmic viscosity and mitotic index were conducted in several mice bearing the ascitic tumor.

TABLE 5.—*Effect of Tissue Extract from CAF_1 Mice upon Mitotic Index of Ehrlich Ascites Tumor Cells in Strong A Mice*

Animal No.	Hours							
	0	5	6	7	8	9	12	24
TP3B-1	1.4	1.2	0.8			1.8	1.8	1.0
TP3B-2	2.2	1.9				1.0		
TP3B-3	1.4	1.1				2.4	1.3	1.8
TP3B-4	1.8	1.9				0.7	1.4	
TP3B-5	2.0	1.4					2.0	1.4
TP3B-6	2.7	3.1					2.7	3.1
TP3B-7	2.4	1.7					2.3	2.0
TP3B-8	1.4	1.5				1.6	1.9	1.4
TP3B-9	2.2	1.7	2.3	2.0	1.8	2.2		2.2
TP3B-10	2.8	1.8	2.3	1.4	1.8	1.6		2.6
TP3B-11	1.7	2.0	2.6	1.8	2.7	2.8		2.3
TP3B-12	0.5	0.3	0.4	0.4	0.6			1.3
TP3B-13	1.0	0.4	0.3	0.5	0.5			1.0
TP3B-14	1.5	1.5	1.6	1.8	1.6			2.0
Mean MI	1.8	1.5	1.5	1.3	1.5	1.8	1.9	2.0
Std. Error	0.2	0.2	0.4	0.3	0.4	0.3	0.2	0.2

Results

I. Rate of Tumor Growth in CAF_1 Hybrid and Strong A Mice.—The ratio of tumor weights, CAF_1 to Strong A, is relatively constant in Groups I, II, and III between 9 and 11 days of growth. No significant difference in the growth rate of tumor is apparent between sexes in the strains. Therefore, the results of the tumor weights are pooled and recorded in Table 1. The mean tumor weight in the subcutaneous layer of CAF_1 hybrid hosts is three times greater than in Strong A for the transplants growing from 9 to 11 days (CAF_1 to Strong A ratio of 3.0). It should be stated that after 11 days of growth the tumor in Strong A mice grows exceedingly fast and tends to invade the surrounding tissues. If

the animals are permitted to live, the size of the tumor in CAF_1 hybrid and Strong A mice does not differ greatly just prior to death.

In contrast, intramuscular tumor growth for the durations equivalent to the above does not show the striking difference noted for the subcutaneous site. The mean weights for the tumors grown in the left thigh is 3.3 gm. with S. E. of $\pm$ 0.1 and 2.1 with S. E. of $\pm$ 0.1 for CAF_1 hybrid and Strong A mice, respectively. The mean tumor weight in the CAF_1 hybrids is about one and one-half times that in Strong A hosts (CAF_1 to Strong A ratio of 1.6).

The intraperitoneal growth of the tumor shows no difference in the rate of development of the neoplastic cells for the two host strains. The cube-root growth phase of male CAF_1 hybrid strain is compared to that of male Strong A mice from data previously determined.[2] Figure 1 shows no significant difference in these curves during the entire growth period up to the asymptotic phase.

From the foregoing results it is apparent that the subcutaneous growth of Ehrlich tumor is inhibited temporarily in Strong A mice up to the 11th day of development. The difference is less striking in the intramuscular site, and no measurable difference can be detected between the two host strains when the cells are grown in the peritoneal cavity.

II. Effect on Protoplasmic Viscosity (in Vitro) and Chemical Properties of Tissue Extracts.—A. Effect of Crude Aqueous Extract of TP1A on Protoplasmic Viscosity of Ehrlich Tumor Cells: In vitro tests on peritoneal samples from three Strong A mice show that the exposure to the crude extract for 15 minutes prior to the centrifugation test reduces the mean protoplasmic viscosity value to 0.71. The controls remain essentially unchanged, with a mean VV of 0.98. No further tests or studies were performed with TP1A. The tissue fractions obtained in our subsequent investigations were all tested in vivo, since this in vitro test established the fact that a simple extract from Strong A tissues lowers the protoplasmic viscosity of tumor cells.

B. Chemical Properties of TP3A and TP3B: Cursory qualitative chemical tests on TP3A and TP3B for evidence of sugars and reducing groups include positive reactions obtained with 5-hydroxymethylfurfural, phenol-sulfuric acid, and periodic acid-Schiff reactions. After the hydrolytic action of concentrated sulfuric acid on TP3A and TP3B, positive tests are obtained with Seliwanoff and Molisch reactions for aldo- and ketohexoses. The positive sugar reactions and a positive test for 1,2-glycol linkages in a water-soluble substance which is not dialyzable across a semipermeable membrane (cellophane) suggest that the extracts consist of a polysaccharide or a mixture of polysaccharides.

In passing it should be mentioned that preliminary attempts at paper electrophoresis reveal four fractions having distinctly separate mobilities. The pattern appears to be similar for both TP3A and TP3B. Further work is contemplated along these lines.

III. Comparison of Histologic Reaction to Tumor Growth in CAF_1 Hybrid and Strong A Mice.—The reactions induced in the host tissues by the subcutaneous implantation of the ascitic tumor suspension differ in CAF_1 hybrid and Strong A strains. The outstanding difference lies in the type of fibroblastic response (desmoplasia) in the two strains.

In Strong A mice the fibroblastic activity is noted as early as two days after the inoculation of tumor cells. Simultaneously, fibrinous exudate and slight edema appear, but inflammatory cells are relatively rare. Later the fibroblasts become numerous and begin to orient themselves in ovoid concentric rings around the main tumor mass, forming a pseudocapsule (Fig. 2). The spindle-shaped cytoplasm of the fibroblasts, therefore, lies parallel to the underlying tumor tissue, and collagen bundles become apparent later. The exudative elements of

Fig. 2.—Note the parallel and orderly arrangement of fibroblasts in the Strong A strain. These cells surround the tumor mass beneath and form a pseudocapsule (10 days). The connective tissue is poorly vascularized. The dark-staining cells in the lower portion of the photograph are tumor cells. The muscular layer above is panniculus carnosus.

inflammation are not prominent up to 10 days.

In the CAF_1 hybrid strain the reaction appears to be one of acute inflammation from the start. Marked edema, infiltration of polymorphonuclear leukocytes, fibrin deposition, and hyperemia are much in evidence by the second day. The fibroblastic response is typical of that seen in the development of granulation tissue, with the fibroblasts irregularly interposed among buds of capillaries (Fig. 3). The development of collagen is minimal even at the end of 10 days. Little evidence of concentric orientation of fibroblasts is noted.

The various staining procedures used in this portion of the study did not disclose any striking differences in the tinctorial reaction between tissues of Strong A and CAF_1 hybrid mice.

The reactions observed for animals given injections of silica gel show similar changes. The fibroblastic reactions parallel those described above for Strong A and CAF_1 hybrid mice (Figs. 4 and 5).

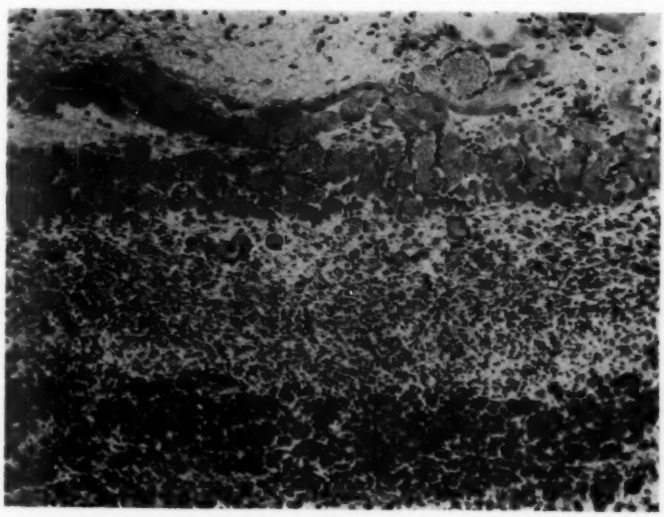

Fig. 3.—Connective-tissue reaction to tumor implantation in the subcutis of CAF_1 hybrid strain (10 days). Note poorly oriented and disorganized fibroblasts amidst numerous buds of capillaries.

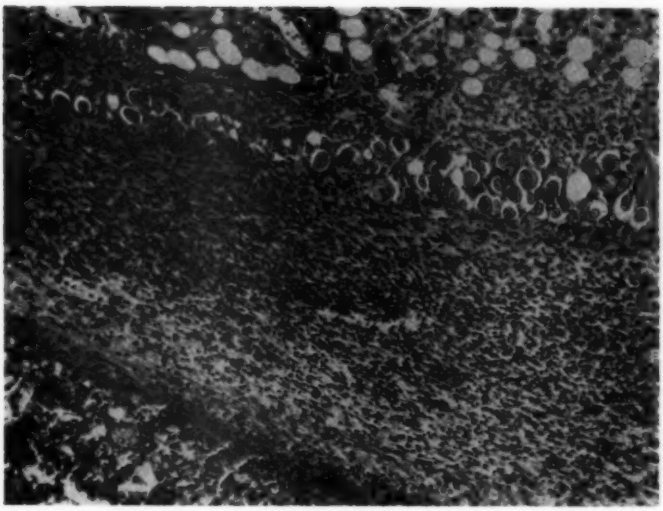

Fig. 4.—Reaction to silica gel in Strong A strain (nine days). The fibroblastic response is very similar to that noted with tumor implantation (Figure 2). The inflammatory reaction, however, is more intense.

IV. Effects of Tissue Extracts on Protoplasmic Viscosity and Mitotic Indexes of Ehrlich Ascites Tumor Cells.—The values for the relative protoplasmic viscosity and mitotic index of tumor cells resulting from the intraperitoneal administrations of TP2A, TP3A, and TP4A are grouped and tabulated in Tables 2 and 3, since each preparation elicited similar responses. The mean viscosity value (MVV) falls rapidly from an initial or base value of 0.97 to 0.62 after a single intraperitoneal injection of the tissue polysaccharide. The MVV remains depressed for about 9 hours and returns to the initial level by 12 hours (Fig. 6). A concomitant rise in the mean mitotic index occurs after a latent period of somewhat less than five hours. After the administration of the tissue polysaccharide from Strong A mice the mean MI with an initial or base value of 1.5 rapidly reaches a peak of 4.5 within seven hours (after the latent period). The mean MI falls rapidly after 7 hours, then more gradually for the next

Fig. 5.—Reaction to silica gel injection in the subcutis of CAF_1 hybrid strain (nine days). Note minimal connective-tissue reaction beneath panniculus carnosus. The mass of silica gel in the lower left-hand corner of the photograph has fallen out of the section during the preparation of the slide.

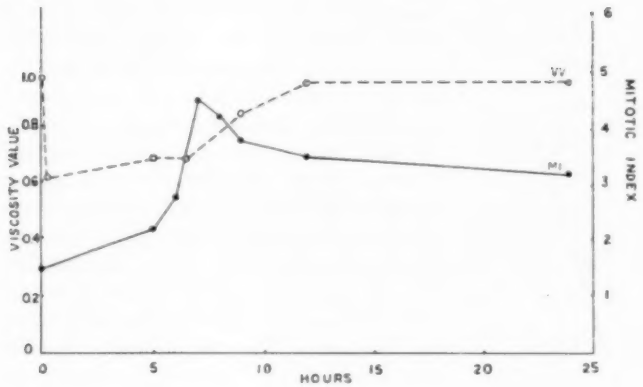

Fig. 6.—The response of tumor cells to the intraperitoneal injection of tissue extracts from Strong A strain. The broken line represents the mean viscosity value, and the solid line, the mean mitotic index.

few hours, but the value does not return to normal even after 24 hours (Fig. 6).

In contrast to the effects noted with the extracts derived from Strong A mice, the product from CAF_1 hybrid mice (TP3B) was inactive when similar tests were performed (Tables 4 and 5, Fig. 7). The viscosity values and the mitotic indexes essentially remain unchanged.

Comment

Physical alterations resulting in the loss of equilibrium of the protoplasmic colloid are believed to be responsible for either the evolution of or the lack of development of the mitotic spindle in the dividing cell.[12] Normal completion of mitosis is dependent, in part, upon the mitotic gelation which ordinarily precedes the formation of spindle fibers.[13] Conversely, metaphase arrest can be induced in the dividing cell by preventing the mitotic gelation, thereby inhibiting the normal development and activity of the spindle. Certain polysaccharides possess the ability to lower the viscosity of the protoplasmic colloid in the living cell and, therefore, can arrest mitosis. Heilbrunn, Wilson, and Harding[3] and, more recently, Heilbrunn and Wilson[14] described extracts from ovaries of many invertebrates and vertebrates (including mammals) which prevent cleavage of fertilized marine annelid eggs. They suggested that the extracts contained antimitotic substances similar to heparin. These preparations were shown also to lower the viscosity of the cell cortex and the inner cytoplasm.

Our own data suggest that the subcutaneous fibroadipose tissue of Strong A mice

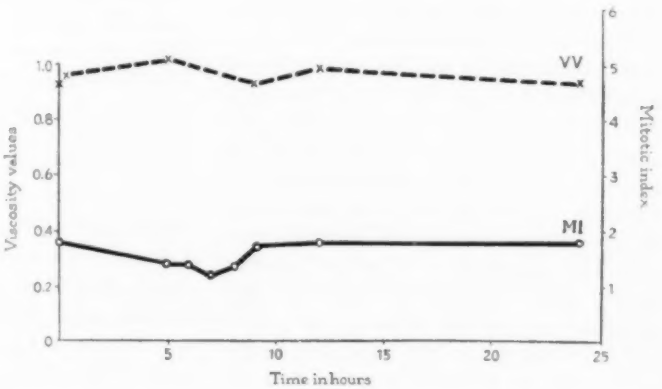

Fig. 7.—The response of tumor cells to the intraperitoneal injection of tissue extracts from CAF_1 hybrid strain. The broken line represents the mean viscosity value, and the solid line, the mean mitotic index.

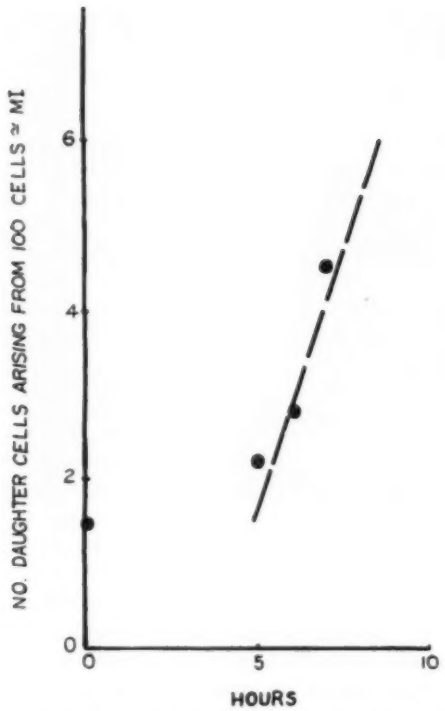

Fig. 8.—Solid circles represent the mean mitotic indexes during the phase in which mitotic figures are rapidly accumulating due to metaphase arrest after a latent period of about five hours. The broken line represents the theoretical multiplication curve of Ehrlich ascites tumor cells at ninth day of growth.

is rich in an antimitotic substance which is not dialyzable and gives chemical reactions for sugars. The data in Tables 2 and 3 and Figure 6 show the correlation between the relative protoplasmic viscosity (VV) and the mitotic index (MI) when mice bearing the ascitic tumor were given intraperitoneal injections of the tissue extracts from Strong A mice. The results are similar to that seen in tests performed with colchicine or Serratia marcescens polysaccharide.[2] These results with the tissue extracts further substantiate the belief that a fall in protoplasmic viscosity precedes mitotic arrest. Figure 8 shows that the rising limb of the MI values, after a latent period of somewhat less than five hours, corresponds to the multiplication rate of tumor cells of about nine days' growth, which represents the average age of the tumor used for the tests. This is an indication that the dividing cells are arrested at metaphase and accumulated at the rate in which the tumor cells normally divide.[2] The percentage of cells in prophase is definitely reduced at the expense of an increasing number of cells in metaphase during the peak point of the elevated MI. The intraperitoneal injection of tissue extract obtained from the CAF_1 strain does not influence either the relative viscosity or the mitotic index of tumor cells sequentially tested (Fig. 7). According to this view the presence of an antimitotic substance, probably a polysaccharide, in the subcutis of Strong A mice will delay the mitotic activity of tumor cells implanted under the skin. It is apparent, however, that the histologic reactivity of fibroblasts differs between the Strong A and CAF_1 strains (Figs. 2 and 3). Whether the presence of the viscosity-reducing carbohydrate-containing substance influences the orderly and concentric arrangement of young fibroblasts in Strong A strain or whether these young cells are responsible for the elaboration of the polysaccharide-like material is not known. It is possible that the action of the viscosity-reducing substance is independent of the regimented fibroblasts. Also it is possible that each of these factors acts separately to delay the growth of the tumor. Although the matter is speculative, the polysaccharide fraction could conceivably act to delay the rate of mitoses of tumor cells while the capsule-like reaction of the connective tissue sets up a physical barrier against the expansion and the growth of the tumor. During the advanced stages of the growth in Strong A mice, Ehrlich tumor transplanted in the subcutaneous layer apparently overcomes the influence of the capsule-like connective-tissue barrier. The invading neoplastic tissue then extends into the muscular layer, whereupon more extensive proliferation may occur—seemingly

from the lack of the restraining factors in this site.

From these observations we conclude that Strong A mice elaborate a mitosis-inhibiting polysaccharide-like substance in the subcutis. This material temporarily delays the proliferation of Ehrlich tumor cells, and the evidence available suggests that it is probably manufactured by the subcutaneous tissue. The presence of tumor tissue apparently is not necessary for the elaboration of the active substance, since silica gel injection also will induce the formation of the mitosis inhibitor by the host tissue of this strain.

Summary

Subcutaneous inocula of Ehrlich ascites tumor developed at different rates in CAF_1 hybrid and Strong A mice, while the rates of peritoneal and intramuscular growths were essentially identical in both strains.

Histologic examination of sections through tumor and surrounding areas revealed differences in the two strains. Strong A mice uniformly showed a characteristic fibroblastic response in the form of a pseudocapsule, which was not observed in CAF_1 hybrid mice.

Chemical extractions of tumors and surrounding subcutaneous tissue from both mouse strains yielded a nondialyzable water-soluble material which was positive to several tests for sugars.

Intraperitoneal injections of the solutions of lyophilized and dialyzed tissue extracts from Strong A strain into mice bearing Ehrlich ascites tumor resulted in reduced protoplasmic viscosity and mitotic arrest of the tumor cells. Such response was not elicited by injection of similarly extracted substance from CAF_1 hybrid mice.

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Response of Breast Cancer to Triethylene Thiophosphoramide

Pathological and Clinical Findings

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In common with other presently known anticancer agents, triethylene thiophosphoramide (TSPA) can be described only as a "mediocre miracle drug." On the credit side can be listed the few patients with certain tumors, i. e., breast and ovary, who have obtained spectacular clinical remissions of their disease followed by months of effective palliation. Such reports (1) are important for the maintenance of a continuing interest and enthusiasm in clinical cancer chemotherapy; (2) give indication of groups of biologically active compounds, derivatives of which may be of greater clinical practicality; (3) add information as to specific differences in the biological response of various tumors, and (4) provide additional conditions under which host-tumor relationships can be studied. The impact upon the patient and family of a favorable clinical response needs no further amplification.

On the debit side must be listed the fatal and near-fatal complications, the small number of patients who respond favorably, the remission of some metastases but growth of others in the same patient, the shortness of the remissions, the failure to cure, and, finally, additive insults to the bone marrow resulting in the prohibition of additional therapy.

The balancing of these evaluations of the usefulness of this and other drugs is further complicated by all of the idiosyncrasies and psychological limitations of a physician attempting to judge clinical phenomena. This study does not represent the best method of evaluating a therapeutic compound but is comparable to a preliminary clinical study of a new agent prior to initiating a definitive clinical trial.

This report includes a brief resume of the clinical experience with TSPA on breast cancer in a small series of patients treated according to specific dosage schedule and evaluated by arbitrary objective criteria.

Triethylene phosphoramide (TEPA) was one of a series of triazines developed by the staff of Lederle Laboratories which showed unusual carcinolytic effects in experimental animals.

The first clinical papers [3,7] describing the effects of this forerunner of TSPA indicated some therapeutic effect upon Hodgkin's disease, the chronic leukemias, lymphosarcoma, neuroblastoma, malignant melanoma, and rarely a sarcoma or undifferentiated carcinoma. The chemical relationship of mechlorethamine hydrochloride nitrogen mustard [methyl-bis (β-chloroethyl)-amine hydrochloride]) triethylene melamine (TEM), TEPA, and TSPA are depicted in Figure 1.

In 1953, Shay and co-workers [5] summarized their first clinical trials with TSPA. In the group of 49 patients with various leukemias, lymphomas, and solid tumors

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Triethylene thiophosphoramide was obtained from Dr. James Ruegsegger, of Lederle Laboratories Division, American Cyanamid Company.

Read before the Section on Pathology and Physiology at the 106th Annual Meeting of the American Medical Association, New York, June 5, 1957.

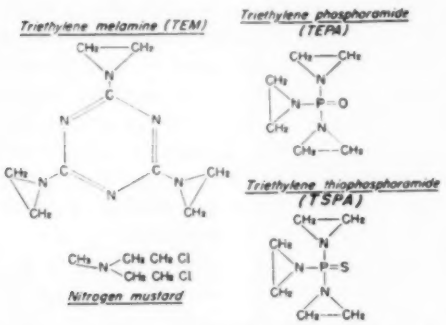


Fig. 1.—Formulae indicating the chemical relationship of several alkylating agents used clinically.

were two patients with recurrent breast carcinoma. Fortuitously the lesions of both patients responded with a disappearance of some lesions and a decrease in the size of others. In a subsequent article [6] Shay and Sun included 12 patients with breast cancer treated systemically with TSPA with varying dosages according to the resulting hematopoietic depression. Six patients showed evidence of objective response which lasted from two months to one year. In most instances daily maintenance therapy had to be discontinued because of hemopoietic depression. Local injection into metastatic lesions was done in two patients with good response even when the lesions were apparently refractive to systemic therapy. Sections of these nodules after treatment were free of tumor cells.

The largest series of breast-cancer patients treated with TSPA have been reported by Bateman. The first paper [1] included 34 patients apparently treated by various routes of administration and given varying dosages but summarized together. Regression of soft-tissue lesions was recorded in 21 of 22 patients, and apparently the majority of these resulted from direct intratumor injections. Control of pleural effusion was effected in 8 of 11 cases, but again the routes of administration were not given.

The intravenous dosage employed was 5 to 20 mg. daily for two to five days, followed by single weekly injections. In the presence of a reduction of white cell count below 5000 the dosage was reduced, and if below 3000, stopped. Degenerative cellular changes were described in nodules in which locally injections were given.

Evaluation of the results achieved by any one kind of therapeutic regimen is impossible from the data given in the paper, since the dosage schedules, routes of injection, criteria of response, and duration of objective remissions are not listed.

In a more recent paper Bateman and Carlton [2] review the clinical findings in 122 breast cancer patients treated with TSPA. Unfortunately, the data in this paper are also indiscriminantly summarized, so that the listing of a 100% favorable response of breast tumors, 72% of metastatic nodules, and 100% of metastatic nodes cannot be evaluated completely. Some of the patients also received adjuvant therapy, such as irradiation or steroid therapy. No duration of remission was indicated, nor was a control series utilized for comparison.

Present Study

On the surgical service of this clinic, approximately 105 patients with advanced malignancy have been treated systemically with TSPA, with use of a rigid dosage schedule. Included in this group were 33 patients with advanced breast cancer which was no longer responsive to steroid therapy and untreatable by local irradiation. The patients eligible for chemotherapy were randomized between a TSPA group and a second group receiving actinomycin D by a blind-envelope technique. If the patient did not respond to the initial compound, the alternate one was attempted after a one-month observation period. A detailed report from this clinic of the effectiveness of TSPA for palliation of breast cancer has been given elsewhere.[4]

Since the purpose of this paper is not a comparison of the two compounds, additional patients not included in the definitive study but treated with the same dosage are included.

Therapy with TSPA consisted of five daily intravenous injections of 0.2 mg. per kilogram, or a total dosage of 1.0 mg. per kilogram. A clinically significant response was defined as a 50% or more reduction in the size of a majority of measurable lesions for a period of one month or more.

Clinical Results

Briefly, 33 patients were entered in the study and 23 completed a full course of therapy (1.0 mg. per kilogram) and lived at least 30 days. Ten patients had a good response according to the criteria listed above, whereas the lesions of the remaining thirteen patients either failed to respond or were reduced in size (but less than 50%) or the period of remission was less than one month. Significant hematopoietic depression occurred in about 40% of the patients, indicating that dosage was nearly the maximum tolerated dose.

The white cell count fell below 3000 in 10 patients, and the platelets, below 100,000 in 11. One patient died from infection as a direct result of her hematopoietic depression. It must be remembered that most of these patients had advanced disease and probably were more susceptible to injury by TSPA as a result of their cachexia and, in some, actual involvement of the bone marrow with tumor.

Pathologic Considerations

A measurable decrease in metastatic skin nodules was observed in two patients less than 72 hours after the first intravenous injection. This, however, was unusual, and ordinarily remissions were first noted about 14 days ± 7 days after completion of the five-day course of injections. Earlier remissions were correlated with the best, most complete responses, and decreases in the size of lesions which became evident subsequent to two weeks after therapy were usually insignificant clinically.

The site of the metastases did not seem to influence responsiveness, with the exception of skeletal metastases, which rarely showed a measurable reduction in size.

Both pleural effusions and abdominal ascites were controlled by systemic therapy in a few cases, but no conclusions as to the relative effectiveness of systemic or intracavity therapy could be made from this study.

The duration of remissions after a single course of therapy lasted from one to three months. Fortunately some of these patients have been maintained in excellent clinical condition for over one year by several subsequent courses of therapy or maintenance therapy consisting of a weekly injection of 0.2 mg. per kilogram, or biweekly, if hematopoietic depression occurred.

The suddenness of the decrease in size and actual disappearance of soft-tissue lesions have been alluded to previously. Unfortunately, the recurrence and regrowth of these lesions appeared just as rapidly. For example, a patient might complain that her widespread skin nodules "popped out like measles during the weekend." Careful mapping of these lesions revealed that the "new" nodules were at identical sites before treatment. In others, however, new lesions occurred, or there was a remission of all but a few nodules, which remained unaffected by the compound.

Of great importance is the observation that in two patients metastatic nodules 1 to 2 cm. in diameter completely disappeared with therapy and have not recurred during the subsequent year of follow-up. Tumor cells cannot be identified at such sites. In one patient no regrowth of some nodules has taken place despite widespread involvement in other areas of the body. Hopefully, such a phenomenon may indicate a complete direct destruction of these specific nodules, since the lack of host resistance seems adequately established by the advancing disease in other areas.

Twenty-four breast-cancer patients had biopsies both before and after TSPA therapy. The remainder either had inaccessible lesions or died before a second biopsy seemed indicated. In eight cases significant microscopic changes were observed. A few additional biopsies revealed noncharacteristic necrosis which could not be evaluated or ascribed to therapy.

Significant changes included areas of bizarre cells, squamous metaplasia, and cytoplasmic vacuolization. Approximately

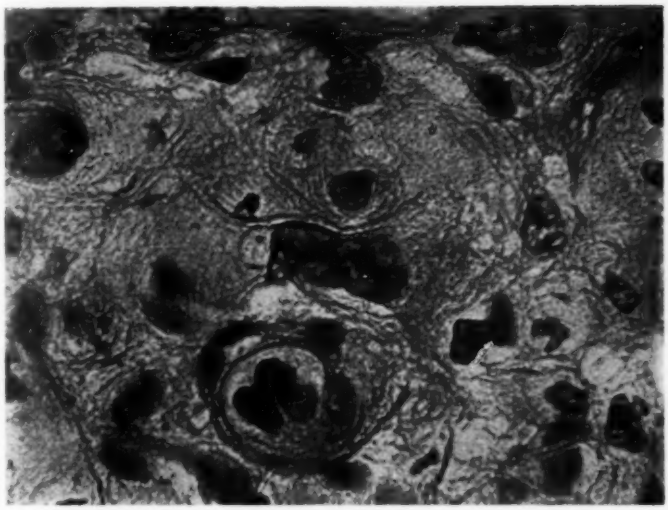


Fig. 2. — Breast carcinoma. Photomicrograph 13 days after therapy. Dosage, 1.0 mg. per kilogram. Note squamous metaplasia.

one-half of the patients showed more than one kind of degeneration.

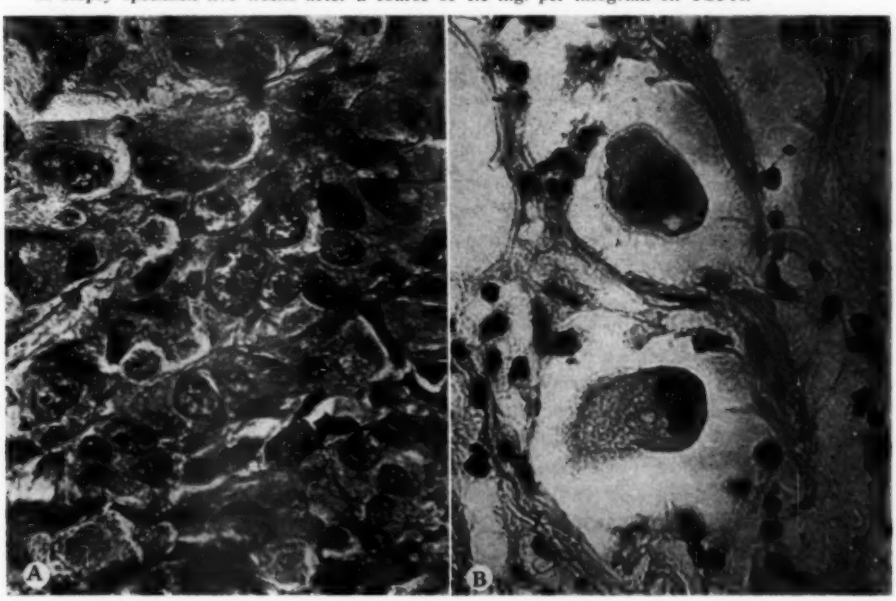
Figures 2, 3*A* and *B*, and 4*A* and *B* depict the contrast in histological appearance of soft-tissue metastasis before and after TSPA therapy.

Abnormal cellular changes were found as early as 72 hours after the first intravenous injection of the compound, as illustrated by Figure 3, and as late as five weeks, as seen in Figure 4.

Strangely enough, the severity of cytological changes was not correlated with the degree of clinical remission.

Biopsies of nodules undergoing rapid diminution often revealed either a complete

Fig. 3.—Second patient. *A*, breast carcinoma before therapy. *B*, representative bizarre cells in biopsy specimen five weeks after a course of 1.0 mg. per kilogram on TSPA.



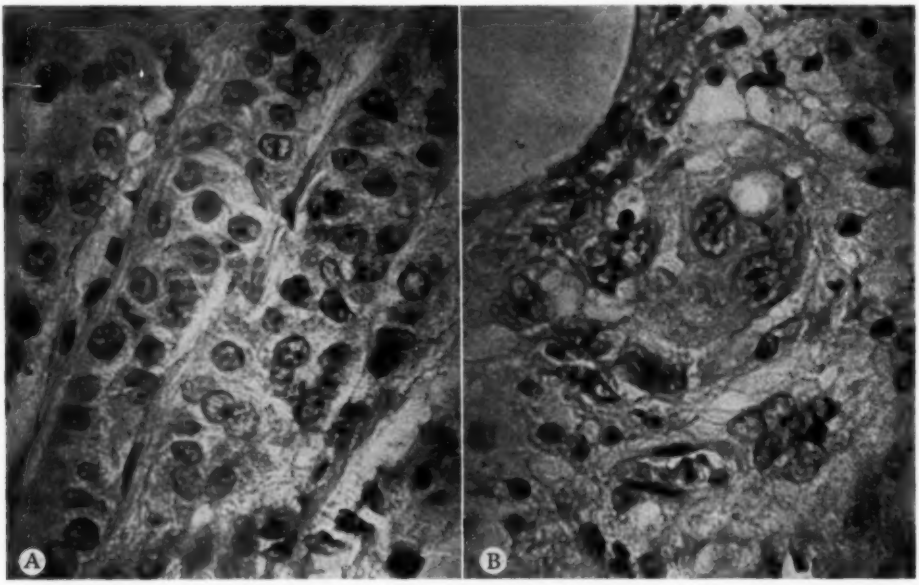


Fig. 4.—Third patient. *A*, breast carcinoma before therapy. *B*, cytological changes two days after first intravenous injection of TSPA. Squamous metaplasia.

absence of tumor cells or a reduction in the number of cells, even though the cells remaining retained a perfectly normal appearance.

Comment and Conclusions

It can be concluded that the systemic therapy of metastatic breast cancer with TSPA effects clinically significant remissions lasting several months in approximately one-half of the patients. The practical limitations of TSPA therapy are a lack of sensitivity of the tumor and toxicity to the bone marrow. General reactions, such as nausea and vomiting, have been mild and infrequent.

The rigid dosage schedule employed in this study undoubtedly resulted in overtreatment of some patients, as reflected by the severe leukopenia and thrombocytopenia encountered, and in undertreatment of others. In general there was no correlation of the extent of hematopoietic depression with tumor regression. If the dosage must be increased until leukopenia occurs in order to obtain tumor regression, a clinical remission cannot be expected, since effective retreatment or maintenance therapy will not be possible.

The gross amount of tumor tissue which can be resolved is often amazing. Huge abdominal and liver metastases identified at surgery have regressed almost completely. In several patients subsequently dying the liver metastases were observed to be deeply umbilicated, and in one instance the gross appearance of a "hepar lobatum" was evident.

Unfortunately, pathologic studies have not given clues as to the mode of action of TSPA or a separation of responsive and nonresponsive lesions. The appearance of giant forms and cells with abnormal mitotic spindles was observed but they are difficult to study. Since study of tumor cells from ascites fluid in untreated patients has revealed a spectrum of chromosome numbers, conclusions as to changes related specifically to TSPA are not possible.

In the introduction it was noted that the present study should not be considered to be an attempt to evaluate the clinical effec-

tiveness of TSPA but the gathering of preliminary clinical data before initiating a definitive clinical trial. The latter can only be properly done in a limited period of time by cooperative groups in various medical centers utilizing a common protocol. Until such studies of statistically significant numbers of patients are completed, the exact status of TSPA in the clinical armamentorium will not be known.

Finally, attention should be called to the fact that cancer chemotherapeutic agents which are effective in causing the regression of advanced tumors may be even more effective if used as adjuvant agents. At the present time, such a cooperative adjuvant chemotherapy study is being initiated. Patients with operable breast cancer will be randomized and TSPA administered during the operation and the first two postoperative days. Thus, the chemotherapeutic agent will be given when the least number of residual tumor cells are present and before host resistance, if any, is lost.

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Host Factors in Fatal Human Lung Cancer

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Investigations of men with lung cancer have pointed to the etiologic importance of the inhalation of various irritants, including arsenic, asbestos fibers, chromates, other metal dusts and fumes, nickel, oils, paints, radioactive pitchblende, siderosilicotic dust, tobacco or other smokes, and wood dust.[1,2] Women with epidermoid lung cancers have probably had significant exposures to cigarette smoke inhalation, but no extrinsic carcinogen has been implicated in women with pulmonary adenocarcinoma.[3,4]

The present study attempts to evaluate the pathologic changes in various host tissues of men and women with fatal lung cancer. Such changes might have some bearing on the individual's susceptibility to develop a pulmonary cancer in the presence of an extrinsic carcinogen or might illuminate the background of lung cancers that develop without significant exposure to any known environmental etiologic agents.

Materials and Methods

A total of 210 autopsied cases of lung carcinoma were collected from four hospitals in the Boston area. Drs. Gustave J. Dammin and Samuel P. Hicks, Peter Bent Brigham Hospital, and Drs. William A. Meissner and Shields Warren, New England Deaconess Hospital, gave permission for material from their files to be analyzed. All available cases were included, except for instances where autopsy limitations precluded a satisfactory study or where insufficient tissues were available for reexamination. Approximately 10% of the cases originally analyzed were discarded for these reasons or because it was concluded that a cancer originally believed to be primary in the lung actually had metastasized to the lung from breast, pancreas, stomach, or kidney. The age and sex distribution of the series is given in Table 1. The lung carcinomas were subclassified as of epidermoid, adenocarcinoma, and undifferentiated types.

TABLE 1.—*Age and Sex Incidences of 210 Autopsied Lung Cancer Cases*

Age, Yr.	Epidermoid		Adenocarcinoma		Undifferentiated	
	M	F	M	F	M	F
30-40	1	1	1	1	2	0
41-50	9	5	5	5	12	0
51-60	27	4	14	9	15	1
61-70	17	1	19	7	20	1
71-81	10	0	5	5	8	2
Total	64	11	44	27	60	4

The analysis included review of the clinical histories, gross autopsy findings including organ weights, and scrutiny of all available microscopic slides, supplemented when necessary by preserved wet-stock tissues. Specimens were usually examined from heart, lungs, spleen, pancreas, liver, gastrointestinal tract, adrenals, kidneys, genitalia, thyroid, parathyroids, lymph nodes, blood vessels, bone, brain, and pituitary gland. Cell counts of the anterior pituitary lobe and testis were made by methods described elsewhere.[5] The data obtained were recorded and tabulated.

The methods used were particularly directed toward the recognition of atrophic or hyperplastic changes in endocrine glands and independently correlated with the pituitary cell counts. This approach has proved useful in analyses of the endocrine abnormalities in the background of and possibly predisposing to cancers of the breast, endometrium, and prostate.[5] These three types of cancer apparently usually develop without the participation of extrinsic carcino-

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gens, and the host tissue changes are relatively common and important. Similar analyses of carcinomas of the uterine cervix and a few autopsied cases of epidermoid skin and lip cancers have showed host changes to be considerably less frequent and presumably less important than extrinsic factors in the background of these particular epidermoid carcinomas. Studies of host factors in malignant hepatomas and stomach cancers, the latter analysis not as yet completed, have suggested intermediate situations involving a host hyperreaction to some irritating factors presumably of exogenous origin. Based on this previous experience with pathologic analyses of host factors in various human cancers, the observations in lung cancer are reported.

Results

The differences found between the host tissue changes in women with pulmonary adenocarcinoma and in the other groups were the most striking. Endocrine abnormalities were very common accompanying adenocarcinoma in women and were considerably less frequent in other groups and types of lung cancer. By inference, extrinsic carcinogenic factors were considered to be generally relatively more important in the background of lung cancer in men than the reactive peculiarities of individual hosts. Because each subgroup was small, no statistical significance is claimed for the findings, which represented observations made with use of simple techniques.

General and Environmental Factors.—Anamnestic and general information is given in Table 2. The observation of rather frequent cancers among the relatives of those with all three types of lung cancer and the high incidence of diabetes in families of women with pulmonary adenocarcinoma are of interest.

Suspected occupations were those involving chronic exposures to substances listed at the beginning of the paper, and 11 machinists were also included because of their likelihood of oil-droplet inhalation. By a heavy use of tobacco was meant cigarette smoking at the level of two packages daily for over 20 years, with some histories indicating that chain smoking had begun at ages of 12 to 14 years. Patients with a suspected occupation and who also smoked cigarettes moderately were separately listed. In support of more extensive epidemiologic research reports, data in Table 2 pointed to extrinsic factors associated either with occupation, habitual tobacco smoking, or both in 94% of men with epidermoid carcinoma of lung, as compared to 49% and 59%, respectively, of men with adenocarcinoma or undifferentiated pulmonary carcinomas.[1,2]

TABLE 2.—*History and Other Diseases Found in Lung Cancer Cases*

	Epidermoid, %		Adenocarcinoma, %		Undifferentiated, %	
	M (64 Cases)	F (11 Cases)	M (44 Cases)	F (27 Cases)	M (60 Cases)	F (4 Cases)
Family diseases						
Cancer	24	20	43	20	33	1
Diabetes	12	10	5	40	11	--
Tuberculosis	4	10	19	10	6	--
Asthma	4	--	5	--	--	--
Occupational suspect	18	--	27	8	14	--
Heavy tobacco use	57	--	15	4	36	--
Occupation & tobacco	19	--	7	--	9	--
Total extrinsic factors	94	--	49	12	59	--
No offspring	38	14	23	37	26	--
Other diseases						
Syphilis	13	--	7	--	5	--
Peptic ulcer	9	9	9	--	15	--
Cholelithiasis	9	--	5	--	8	--
Hernia	8	--	5	--	8	--
Colonic polyp	3	--	5	--	7	1
Diabetes	2	--	2	4	3	--

The Figures in the last column refer to number of cases.

Investigation of marital and reproductive histories showed that single persons or those with sterile marriages were commonest among men with epidermoid carcinomas and women with adenocarcinomas of lung. Other major diseases reported did not seem disproportionate, except for peptic ulcers and the frequency of syphilis accompanying epidermoid carcinoma. This reflected a possible synergistic effect of syphilis upon the development of some pulmonary cancers, like that already recognized as true of carcinomas of the tongue and uterine cervix.

In Table 3 are shown items from the histories of men with lung cancers, indicat-

TABLE 3.—*Social History and Accidents of Men with Lung Cancers*

	Epidermoid, %	Adenocarcinoma, %	Undifferentiated, %
Separated, divorced or remarried	12	19	12
Venereal disease	16	7	12
Alcoholism	9	3	14
Traumatic fractures, scars, and amputations	14	5	12

ing rather frequent serious marital problems, venereal diseases, admitted alcoholism, and severe trauma, with scars, fractures, or amputations. These events were not all localized to a few persons but suggested a group peculiarity of the men with lung cancer. Taken with the not inconsiderable frequencies of hernia and peptic ulcers, an impression was gained of some men with excessive nervous energy that often was expressed to their physical detriment. These men had overstrained their physical capacities in various ways, as judged by the collected data. The same situation was not found in the women with lung cancer and has not been observed to characterize other autopsied cancer-site case series similarly studied.

Lung Factors.—Previous pulmonary diseases found are listed in Table 4. Care has been taken to exclude inflammatory or degenerative conditions that likely arose secondary to the lung cancers. The highest incidences of recognized antecedent clinical and pathologic bronchial diseases, 28% and 78%, respectively, were found accompanying epidermoid carcinoma, and the highest incidences of predominantly parenchymal lung diseases, 38% and 18%, respectively, accompanied undifferentiated carcinomas. Chronic irritation of bronchial surfaces was somewhat commoner prior to the development of epidermoid carcinomas than other lung cancers. Fibrosis following parenchymal lung injury was seen more frequently antedating undifferentiated lung carcinomas, and some instances of scar cancer (Narbenkrebs) were included in this group.[6,7] In adenocarcinoma cases there were intermediate values for both chronic bronchial diseases and pulmonary fibrous scarring.

Among the less common chronic bronchopulmonary diseases antedating lung cancers in this series were three cases of bronchial asthma and two of bronchiectasis in men and two cases of bronchial asthma and two of bronchiectasis in women preceding the development of epidermoid carcinomas. There also were two men who had bronchiectasis followed by undifferentiated carcinomas and one woman with bronchiectasis complicated by a pulmonary adenocarcinoma. Histories of significant occupational exposures or smoking were generally not obtained in this group. These 12 cases lent support to the belief that a variety of chronic bronchial and parenchymal lung

TABLE 4.—*Prior Pulmonary Diseases in Lung Cancer Cases*

	Epidermoid, %		Adenocarcinoma, %		Undifferentiated, %	
	M (64 Cases)	F (11 Cases)	M (44 Cases)	F (27 Cases)	M (60 Cases)	F (4 Cases)
Clinical						
Chronic bronchitis	11	..	8	..	6	..
Pneumonia	15	10	19	8	26	1
Asthma	7	10	5	4	..	..
Influenza	7	10	8	..	3	..
Tuberculosis	5	..	5	..	9	..
Bronchiectasis	3	9	3	..	..	..
Emphysema	2	..	..	..	..	..
Empyema	2	..	..	..	3	..
Pathologic						
Bronchi						
Hyaline fibrosis	45	9	35	23	32	2
Epithelial hyperplasia	25	25	23	8	12	..
Squamous metaplasia	14	9	16	4	5	..
Pulmonary fibrosis	2	..	9	11	18	..
Pneumoconiosis	5	..	2	4	..	..

The Figures in the last column refer to number of cases.

TABLE 5.—*Major Endocrine Changes in Women with Lung Cancer*

	Adenocarcinoma (27 Cases), %	Epidermoid & Undifferentiated (15 Cases), %	No Cancer (248 Cases), %
Hyperplasia of			
Ovarian stroma	88	58	37
Breast (fibrocystic)	67	60	28
Pituitary HA cells *	86	80	5
Endometrium	62	64	27
Pancreatic duct	31	0	6
Adrenal cortex	24	7	4
Thyroid atrophy	28	22	11
Nodular	28	44	11
Adenomatous goiter	20	11	6
Normal	24	22	65
No endocrine changes	11	19	63

* HA indicates hypertrophic amphophilic.

diseases may occasionally predispose to carcinoma.[8,9]

Endocrine Changes in Women.—As shown in Table 5, hyperplastic overgrowth of ovarian cortical stroma characterized 74% of the entire female group with lung cancer and was most striking (88%) accompanying pulmonary adenocarcinoma. Ovarian stromal hyperplasia has been found in about the same proportion of women with carcinomas of the breast and endometrium.[10] Biochemical synthesis experiments in vitro with use of human ovarian stromal tissue have demonstrated that it produces estrogens labeled with radioisotopes.[11,12] Estrogen effects in older women were evident in their cervical epithelium, and in the present series about two-thirds of the endometrial and breast specimens showed hyperplastic or cystic overgrowths of types ascribable also to estrogenic stimulation.

Pituitary-adrenocortical hyperfunction of a particular type, characterized by increased numbers of anterior-lobe hypophyseal cells termed hypertrophic amphophils (HA cells), was the other most frequent endocrine change observed. Hypertrophic amphophil pituitary cells were first described as accompanying a variety of human adrenocortical hyperplastic syndromes.[13] These cells have been found in increased numbers accompanying actively growing cancers of several internal organs. In the present series a pronounced adrenocortical hyperplasia was found in 24% of women with pulmonary adenocarcinoma. Metastases interfered with the analysis of sufficient cases of other types of lung cancer in women.

Pancreatic-duct hyperplasias are as yet of uncertain etiology, although similar changes have occurred in animals treated with growth hormone. The incidence of 31% pancreatic-duct hyperplasia hinted at participation of other hormonal activities in the growth of pulmonary adenocarcinomas in some women, possibly including pituitary somatotropin (STH, or GH).

Thyroid abnormalities of various types were frequent in women with lung cancer. This appeared attributable to some relationship between ovarian and thyroid activities that has not yet been elucidated despite many investigations. In a minority of women with lung cancer no host endocrine abnormalities were found, and the pathogenesis remains without explanation.

Endocrine Changes in Men.—In males with lung cancers, unlike females, there were commonly indications, both clinical and pathologic, of long-continued chronic irritation of bronchopulmonary tissues. Endogenous changes were hidden or only vaguely discernible behind this smoke screen of environmental factors. Another serious limitation of analysis was the relatively less extensive detailed knowledge of endocrine pathology in men. Some criteria of estrogenic and somatotropic effects in older men are recent, unconfirmed, and not well known.[5] With these shortcomings recognized, an attempt is made to summarize the findings in Table 6.

As in women, men with adenocarcinoma had the most frequent indications of endocrine abnormalities, chiefly in the sphere of pituitary gonadotropic basophils and immature testicular tubular cells, found to be enlarged and increased in situations associated with augmented or uninhibited estrogen in men.[5] All types of lung cancer in men showed morphologic evidences of inferred pituitary-adrenocortical hyperactivity,

TABLE 6.—*Major Endocrine Changes in Men with Lung Cancer* *

	Epidermoid (64 Cases), %	Adenocarcinoma (44 Cases), %	Undifferentiated (60 Cases), %	No Cancer (217 Cases), %
Hyperplasia of				
Pituitary HA cells	80	90	90	2
Pituitary Bas cells	30	60	60	14
Pancreatic islets	25	27	24	6
Immature spermatogenic cells	18	48	25	8
Adrenal cortex	22	7	15	4
No endocrine changes	34	18	23	60
Abundant Leydig cells	61	45	28	43
Overt endocrine changes	11	40	22	16

* HA indicates hypertrophic amphophilic; Bas, basophilic.

TABLE 7.—*Average Pituitary Cell Counts in Lung Cancer and Control Cases* *

	No. Cases	Sex	Cells	Ac	Bas	Amp	Chr	HA	HB
Epidermoid carcinoma	10	M	10,332	33.5±5.1	21.4±5.5	19.0±5.1	24.0±3.7	2.0±2.5	<0.1
Adenocarcinoma	10	M	10,321	29.9±7.0	23.4±6.4	21.7±6.5	22.7±3.6	2.3±0.4	<0.1
Undifferentiated	10	M	10,356	31.5±5.1	23.4±7.4	20.1±7.4	22.9±2.2	2.0±0.5	0.1
Control	10	M	10,136	40.8±7.0	17.8±2.4	19.1±5.9	21.5±1.3	0.8±0.4	<0.1
Adenocarcinoma	9	F	10,337	34.4±7.7	15.8±7.2	16.9±7.1	21.0±5.3	1.7±0.9	0.1
Epidermoid-undifferentiated	5	F	10,135	32.4±4.1	21.9±6.4	20.9±5.8	22.3±1.8	2.3±0.9	0.1
Control	10	F	10,470	37.4±5.3	17.6±2.8	22.4±5.5	21.5±3.1	0.9±0.1	<0.1

* Ac indicates acidophils; Bas, basophils; Amp, amphophils; Chr, chromophobes; HA, hypertrophic amphophils; HB, hyaline basophils.

as is true of various other malignant internal neoplasms.[14]

When endocrine alterations exclusive of the pituitary counts were correlated in men with pulmonary adenocarcinomas, about 40% showed overt changes, almost equally divided into suggestions of estrogen excess, hints of pituitary growth hormone effects such as pancreatic-islet hyperplasia and visceromegaly, or combinations of inferred estrogenic and growth hormone stimulation. Men with undifferentiated lung carcinomas showed overt endocrine changes in 22% of cases, approximately equally divided between estrogenic and growth hormone effects or evidence of combinations. Eleven per cent of men with epidermoid carcinomas had similar abnormalities.

Pituitary Cell Counts.—The averages given in Table 7 provided independent evidence of an increased activity in a majority of men with lung cancers of all types. Women with adenocarcinoma also showed evidences of anterior pituitary lobe hyperactivity. Insufficient cases were available to establish any statistical validity, but a comparison with counts from noncancer control cases of comparable ages suggested real differences.

Hypertrophic amphophil cells, thought to reflect a particular type of pituitary-adrenocortical hyperfunction,[13] were increased in practically all the pituitary glands of the lung cancer cases investigated, except for a few complicated by intracranial metastases. Hyperplasias of hypophyseal gonadotropic basophil cells were found in 6 of 10 men with adenocarcinomas and 3 of 10 men with epidermoid carcinomas. Of nine women with pulmonary adenocarcinomas, two had increased pituitary gonadotropic basophil cells, as did two of five women with other types of lung cancer.

Acidophils were increased, reflecting possible growth hormone activities, in pituitary glands of about 10% of men with lung cancer and were hyperplastic in three of eight women with adenocarcinoma. All pituitary cell counts were made without knowledge of the sex or type of cancer involved.

Multiple Cancers.—Thirteen men with epidermoid lung cancers had 15 other primary cancers, including 4 malignant lymphomas, 3 adenocarcinomas of gastrointestinal tract, 3 adenocarcinomas of prostate, 2 epidermoid carcinomas of the oral cavity or upper respiratory tract, 1 double primary renal-cell carcinoma, and 1 each epidermoid carcinoma of anus and myelogenous leukemia. Two women with pulmonary epidermoid carcinoma also had a breast carcinoma, and one each had carcinomas of vulva and stomach. Four men with pulmonary adenocarcinomas had other cancers, including two prostatic adenocarcinomas, a facial basal-cell carcinoma, astrocytoma, and ampullary adenocarcinoma of duodenum. Five women with lung adenocarcinomas also had other neoplasms: two, adenocarcinomas of colon; one, adenocarcinoma of endometrium, a mammary fibrosarcoma, and an epidermoid carcinoma of cervix. Seven men with undifferentiated lung carcinomas had other primary cancers: four, prostatic adenocarcinomas; two, oral epidermoid carcinomas, and one, myelogenous leukemia. One woman of this group had a separate mammary carcinoma, and one woman, a skin cancer.

Comment

Analysis of the collected data on lung cancer has been undertaken with a realization that a retrospective pathologic study of autopsied cancer cases naturally introduces various selective factors and is not necessarily pertinent to the findings in a randomly selected or unselected population of the living. However, understanding of many medical problems has progressed from a prior recognition at autopsy of more extensive and extreme disease manifestations to a subsequent uncovering of comparable earlier and less obvious processes in the living. Techniques of pathology are adaptable to investigations of host-environmental relationships in human cancer. Space limitations do not permit citing all the important recently published epidemiologic, clinical, and pathologic reports dealing with lung cancer. Certain factors, such as urban atmospheric pollution, were not accurately assayed in the present study.

Two major subgroups of lung cancer hosts have been distinguished. One is exemplified by women with pulmonary adenocarcinomas, who had a fairly common history of familial diabetes mellitus or cancer. This background of familial neoplastic and metabolic diseases was also found to be rather frequent among lung cancer cases as a whole and might suggest a genetic trend. Present information did not answer the question whether these persons were unusually disposed to develop cancers, which were localized in lung owing to environmental exposures.[15] The 17% incidence and the types of other primary cancers found did not support this theory, since cases of multiple cancer in which the lung is one site are not frequent, in comparison with those in which some other organs are involved.[5]

Endocrine host alterations were prominent in women with adenocarcinoma, and significant environmental etiologic factors were uncommon. The pathologic changes usually included ovarian stromal hyperplasia with an inferred continuous estrogen secretion. Bronchial glands and bronchiolar epithelium were thought likely to have responded by the development of hyperplasias and ultimately adenocarcinomas. Whether or how often the antecedent hyperplasia took the form of a benign "pulmonary adenomatosis" could not be determined from the present material.[16]

Experimental and clinical evidence previously collected has indicated that estrogenic hormones in the first stage of carcinogenesis primed the tissues of ovarian target organs through the stimulation of epithelial hyperplasias and that unless a further pituitary-adrenal endocrine abnormality subsequently developed carcinomas were not produced.[10,17]

In the limited material collected from women with pulmonary carcinoma, the same situation has seemed to prevail. Pituitary-cell counts and other endocrine alterations were interpreted to indicate a remote state of hyperestrinism, usually extending into the postmenopausal period, succeeded by a pituitary amphophilic-cell hyperplasia and either an obvious morphologic or an inferred functional adrenocortical hyperactivity. Neither the pituitary nor the adrenal hormones involved have been clearly identified yet. The inferred pituitary-adrenal hyperfunctional state associated with these morphologic changes has been thought to comprise the second stage of carcinogenesis, during which cancers grew out from tissue hyperplasias such as those produced by estrogen.[17] Cell counts have suggested an abundant pituitary growth hormone production in some of these cases.

Men with pulmonary adenocarcinomas had stigmata of a comparable estrogen-induced glandular hyperplasia and a later pituitary-adrenal stimulation of cancer outgrowth in about 25% of the cases studied. The male endocrine changes require further investigation and confirmation. About 50% of men with adenocarcinomas had significant environmental exposures to suspected lung carcinogens, and in the remaining 25% effects of growth hormone, possibly of pituitary origin, were often inferred.

Epidermoid lung cancers composed the other major entity, occurring predominantly in men. Again there were fairly frequent family histories of cancer, and the reproductive activity was subnormal. These observations might reflect either genetic factors or a fertility reduced by environmental influences. Whether the indications of various social and possibly related medical difficulties in some of the men with lung cancers reflected genetic, hormonal, psychologic, or psychiatric abnormalities would require further study.

Over 90% of the men with epidermoid lung cancers had significant histories of chronic exposures to suspected respiratory carcinogens. Occupation was implicated in 37%, and heavy indulgence in cigarette smoking in 57%. Most of the remaining 6% had asthma or bronchiectasis. Bronchi of the uninvolved portion of lung showed a submucosal hyaline fibrosis, usually with chronic inflammation and often with epithelial hyperplasia, more commonly than occurred with other types of lung cancer. Bronchial epithelial hyperplasias accompanying lung cancer are observed significantly more often if specifically and systematically sought.[18]

Squamous metaplasia of bronchi did not occur frequently enough or in locations that indicated any direct relation to cancer development, in agreement with some European reports.[19]

Endocrine abnormalities, except for inferred pituitary amphophil and adrenocortical hyperfunctional states, were uncommon accompaniments of epidermoid carcinomas in men. "Maleness" as an important factor in the development of bronchogenic carcinomas was reflected by the maintenance of abundant testicular Leydig interstitial cells in a majority of men with epidermoid carcinomas, while the other cancer groups and controls showed the more usual tendency to interstitial-cell atrophy with age. The hint was that virilizing hormonal activity and effects were more prominent in older men who developed epidermoid lung carcinomas. Androgens are not generally regarded as cocarcinogenic, and the hormonal influences involved may have been expressed in masculine habits that increased environmental exposures.

If the two-stage hypothesis of carcinogenesis described above is acceptable, one may consider that preceding bronchogenic epidermoid carcinomas the first-stage hyperplasias of bronchial epithelium developed as a host reaction to one or more air-borne irritants, usually chemical but sometimes bacterial or allergenic. Chronic inflammatory and fibrotic bronchitis would be expected to occur in most persons with comparable exposures. Host factors responsible for associated epithelial hyperplasias and metaplasias in some persons would need additional study. Outgrowths of epidermoid lung cancers, insofar as they were dependent on the second-stage pituitary-adrenal changes, occurred only in the persons who developed this endocrine alteration subsequent to sufficient environmental exposures.

Animal experiments have suggested that increasing age and a decline in the gonadal functions of either sex were closely associated with establishment of this particular pituitary-adrenocortical hyperfunctional state. Extrinsic carcinogens accelerated the onset of this change, which otherwise might naturally ensue later.[17] Incidentally, pituitary amphophil and adrenocortical hyperactivities that developed in animals lacking an established tissue hyperplasia did not promote cancer outgrowths.

Undifferentiated carcinomas formed an intermediate group, somewhat more closely resembling adenocarcinomas, as Weller[20] pointed out. With undifferentiated carcinomas, indications of antecedent localized or diffuse pulmonary fibrosis were commoner. Fibrous scars from tuberculosis, pneumoconiosis, organized pneumonias, or idiopathic diffuse fibrosis were more often associated with the outgrowths of an undifferentiated type of cancer.[6,7] Endocrine

abnormalities, when present, resembled those already discussed.

Too few cases of women with epidermoid or undifferentiated carcinomas were studied for an accurate appraisal of their pathogenesis.

Summary

A pathologic study of 210 autopsied cases of lung cancer, with particular attention to host factors, is reported. Women with pulmonary adenocarcinoma commonly showed indications of a prior hyperestrogenic state with ovarian stromal hyperplasia, succeeded by a particular type of pituitary-adrenocortical alteration and an inferred endocrine hyperactivity associated with cancer outgrowths. Environmental factors appeared to be of minor importance.

Men with epidermoid lung carcinomas usually had histories of prolonged exposures to respiratory irritants and commonly had developed chronic bronchial inflammation and fibrosis, with or without epithelial hyperplasia. Host endocrine changes with established epidermoid carcinomas usually included an inferred pituitary-adrenocortical hyperfunctional state. Undifferentiated lung carcinomas comprised an intermediate group in regard to the influence of environmental and host factors and developed more often in lungs with antecedent fibrous scarring.

The observations are discussed in relation to a two-stage hypothesis of carcinogenesis.

60 E. Newton St. (18).

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News and Comment

PERSONAL

Election of Dr. Howard T. Karsner.—Dr. Howard T. Karsner, medical research advisor to the surgeon general, was elected honorary president of the International Society for Geographic Pathology at the sixth conference of the society, in Paris, July 9 through 11. This nongovernmental group aims to study the relations which might exist between diseases and the geographical areas in which they occur.

Appointment of Dr. Edward A. Gall.—Dr. Edward A. Gall, head of the pathology department, University of Cincinnati College of Medicine, has been named editor in chief of *The American Journal of Pathology.*

Appointment of Dr. Robert A. Moore.—Dr. Robert A. Moore has been appointed president of the Downstate Medical Center of the State University of New York and dean of the College of Medicine of the Center. Dr. Moore for several years has been vice-chancellor of the health professions at the University of Pittsburgh.

Award for Dr. Richard E. Shope.—Dr. Richard E. Shope received the Kober medal of the Association of American Physicians at the annual meeting, in recognition of his work in the field of animal viruses.

ANNOUNCEMENTS

Institute for Study of Congenital Heart Disease.—A Congenital Heart Institute sponsored by the Chicago Heart Association was started Sept. 1 and will continue for a minimum of 10 years. Dr. Maurice Lev has been hired as full-time investigator to devote all his time to a study of the life history and pathology of the defects of the heart. Dr. Lev will perform his investigative work in laboratory and working space provided for him in the Hektoen Institute of Chicago, and the work will be done under the cooperation of the five Chicago medical schools, the Chicago Heart Center, and the health agencies of city and state. Dr. Lev plans to assemble clinical, laboratory, and pathologic data on cases of children who die of congenital heart defects. Cost of the institute will be about $50,000 a year.

Advanced Training Neurological and Sensory Disorders.—The Public Health Service today announced a new program of financial support for advanced training of research scientists in the field of neurological and sensory disorders.

The new program, designed to help research scientists obtain additional specialized training for careers in teaching or research, will be conducted by the National Institute of Neurological Diseases and Blindness, of the Service's National Institutes of Health, Bethesda, Md.

A previous program, under which about 75 scientists received advanced training during the last fiscal year, was concerned exclusively with clinical training. The program announced today is designed to encourage advanced training in either the clinical area or in such basic science areas as neurochemistry, neuropharmacology, neurophysiology, or neuroanatomy.

Individual awards under the program generally will be made for not less than nine months and for not more than one year. However, all awards are subject to renewal and may be continued for a period of three years. Stipends are determined individually in accordance with each applicant's qualifications and training needs. Such stipends may range from $5500 to $14,800 a year.

Application forms and instructions may be obtained by writing to the Chief, Extramural Programs Branch, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda 14, Md. Completed applications should be submitted to the same address.

Applicants must be citizens of the United States or must have filed declarations of intent to become citizens. They must be free of any physical or mental disability that would interfere with the proposed training. In addition, they must have completed either (1) the residency training requirements in a clinical specialty or its equivalent or (2) at least three years of pertinent postdoctoral training or research experience.

Special traineeships may be awarded for training at any institution in the United States or abroad qualified to give the particular training desired by the applicant.

The trainee may begin training at any time within 10 months of the date of his award. During the period of training the trainee will not be permitted to carry on the private practice

of medicine; he may engage only in such teaching, clinical, and research activities as are a prescribed part of his training program.

Training Seminar in Diagnostic Use of Radioisotopes.—This seminar is sponsored by the American Society of Clinical Pathology and the Oak Ridge Institute of Nuclear Studies and is to be held at the Medical Division, Oak Ridge, Tenn.

A training seminar on the diagnostic use of radioisotopes has been set up specifically for certified pathologists and designed to meet the recommendations and requirements of the Atomic Energy Commission. This seminar will furnish a reasonable introduction to radioisotopes for those pathologists who are principally interested in diagnosis but who may be called on to assist and advise on therapeutic uses of radioisotopes. The program consists of two one-week meetings separated by a three-month interval. The first (basic) week consisted mainly of fundamental techniques and concepts. The second (clinical) week will be held three months after the first and will be devoted mainly to the practical application of these principles. It is expected that in the intervening three months the participating pathologists will visit laboratories in their home areas which are already approved for the use of isotopes and will assimilate relevant literature. At the end of the second week the Medical Division of ORINS will give to participants a statement of preceptorship in the diagnostic uses of radioisotopes. It is emphasized that while this does not automatically qualify any person for AEC permission to use isotopes, the course is designed to meet the training requirements of the Isotopes Extension of the AEC.

The training seminar will consist of approximately 50% lectures and demonstrations and 50% laboratory work. Each participant will be expected to administer to himself a dose of less than 10μc. of I^{131} to accomplish a thyroid-uptake or a blood-volume measurement.

The teaching staff has been selected from pathologists, internists, surgeons, radiologists, physicists, and biochemists in and around Oak Ridge or members of the Council on Radioisotopes, ASCP. All are experienced in the clinical use of radioisotopes.

The first one-week program was held Nov. 11-16, 1957; the second week will be held Feb. 10-15, 1958.

Requirements: Applicants must be certified in either Clinical Pathology or Pathologic Anatomy or be members or fellows of the American Society of Clinical Pathology, must be United States citizens, and must have a license to practice medicine. Applications will be reviewed by the Council on Radioisotopes, ASCP. Qualified candidates will be accepted in chronologic order.

Housing: For assistance in obtaining housing, write to Dr. Harold Steffee, Oak Ridge Institute of Nuclear Studies, P. O. Box 117, Oak Ridge, Tenn.

Fee: Entire course, $25, payable to the Oak Ridge Institute of Nuclear Studies on the first Monday.

Application: Application for this training seminar should be made to Dr. Oscar B. Hunter Jr., Chairman, Council on Radioisotopes, American Society of Clinical Pathology, Suite 1000, Columbia Medical Building Annex, 915 19th St., N. W., Washington 6, D. C. The first training seminar will be limited to 16 pathologists.

Tentative Program for Second Week:

	Morning	Afternoon	Evening
Monday	Thyroid Rounds	Iodine Measurements	Social
Tuesday	Blood Volume Measurements	Thyroid Scans	Clinical Conference
Wednesday	Electrolyte Spaces and Clinical Rounds	Counting Methods	Thyroid Uptake Laboratory
Thursday	Autopsy Techniques	Autoradiography Interpretation	Clinical Conference
Friday	Clinical Rounds and Renograms	Therapeutic Use of Isotopes	Measurements Laboratory

Time: The training seminar will meet each day 9:00 a.m. to 1:00 p.m., 2:00 p.m. to 6:00 p.m., and 8:00 to 10:00 p.m. An organizational meeting will be held at 8:00 a.m. on Monday of each week.

Books

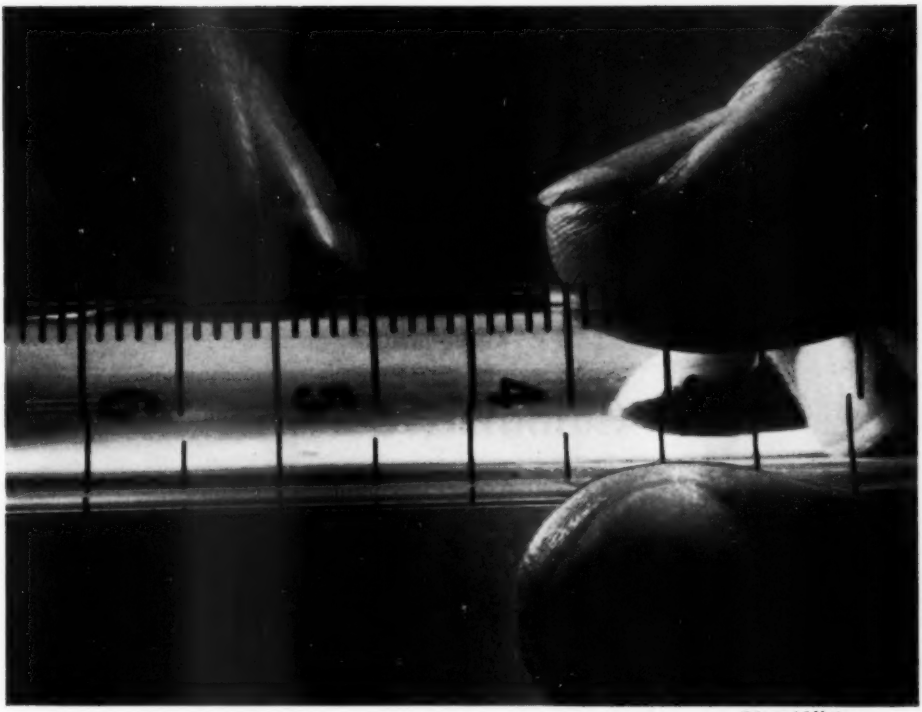
Lehrbuch der speziellen pathologischen Anatomie. Band II-1. Eleventh and twelfth edition. By E. Kaufmann. Edited by M. Staemmler. Price, DM 62. Pp. 404, with 148 illustrations. W. de Gruyter, Genthiner Strasse 13, Berlin W. 35, 1957.

This book constitutes the first part of the second volume of the "new Kaufmann" edited by M. Staemmler. It deals with the male and female genital tracts and is written by H. Gogl and F. J. Lang. Twenty pages are devoted to malformations in general. Every phase of disease is discussed in detail, often with statistical data giving incidence and age range. The authors refer to seminomas and pseudoseminomas instead of using our commoner nomenclature of dysgerminoma. They describe "histologically benign" and "histologically malignant teratomas." However, it is stressed that "biologically" some of the former may be malignant and metastases of these tumors may show outspoken carcinomatous or chorioepitheliomatous elements. Included among the "histologically malignant teratomas" are teratocarcinomas and choriocarcinomas. There is a clear description of the gross appearance and microscopic features of the various ovarian tumors but only fragmentary discussion of the pathophysiology of these tumors. The carcinoma of the cervix and body of the uterus is taken up in great detail, but very little mention is made of diagnosing these tumors by exfoliative cytology.

In general, the book is well written, and the German is clear-cut and easily understandable. Some of the 148 illustrations are out of focus, but the majority are of good quality. This volume can be well recommended for these pathologists and clinicians who concentrate their efforts in this speciality. Particularly those seeking opinions of long past authorities will be gratified by the extensive references, many of which date back to the early part of this century and some even to the last century. This reviewer would have preferred a treatise based on present personal experience of the authors principally, only with up-to-date literature and much more reference to pathophysiology and clinical medicine. As it stands, however, it presents an excellent volume of the old-fashioned pathologic anatomy of the genital tract system.

The Leukemias: Etiology, Pathophysiology, and Treatment. By John Rebuck, Frank Bethell, and Raymond Monto. Price, $13. Pp. 711, with 308 illustrations. Academic Press, Inc., 111 Fifth Ave., New York 3, 1957.

This book is a result of the International Symposium on Leukemia, held at the Henry Ford Hospital in Detroit, in March, 1956. The participants were nationally and internationally eminent hematologists, biochemists, and leukemia investigators, some of whom have contributed to the field of leukemia research for many years. This book is a compilation of the papers presented at that symposium, together with the commentaries on the papers by the participants. The book does not cover the entire field of leukemia but limits itself to current experimental work and findings in laboratory and human leukemia as well as basic work about leukocytes. A wide range of subjects are covered, which include the submicroscopic structure and antigenicity of leukemic cells, genetics and immunogenetics of mouse leukemia, physiology and biochemistry of leukocytes and leukemic cells, as well as chemotherapy and mechanisms of drug action. The book ends with a round-panel discussion, with internationally famous hematologists participating, on current trends and concepts in human leukemia therapy. The material is all current and is of greatest interest to the student of leukemia. Many of these papers, or others in similar form, have been published previously by these investigators. The great value of this book is the fact that all of these current papers on basic leukemia research are gathered together in one volume.



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3. Bowden, F. P.: **Jour. Proc. Roy. Soc. N. S. Wales** 78:187, 1945.

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